STUDIES OF RINDERPEST VIRUS IN PREGNANT ANIMALS

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DEDICATION

To my father for his inestimable contribution to my education and to my wife Susan and children for their enduring patience and love

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DECLARATION

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ABSTRACT

A critical review of the literature back to the 18th century revealed that abortion in cattle infected with rinderpest virus has received scant attention despite a handful of early reports of its occurrence. These early reports have been confirmed; three strains of the virus of varying degrees of virulence induced abortion in cattle while attenuated vaccine strains did not. Over 50 per cent of the abortions occurred 2 to 7 weeks following clinical recovery and most foetuses aborted at this stage showed virological and antigenic evidence of *in utero* transplacental infection with the virus. Foetuses aborted during or soon after the acute stage of the disease had no demonstrable evidence of transplacental infection. Some cows did not abort and delivered normal calves at term, a few of which had pre-colostral serum neutralizing antibodies against rinderpest virus indicating *in utero* infection.

The experimental results were reinforced by a natural attack of rinderpest in the field in which there was a high incidence of abortion in cattle 2 to 4 weeks after the disappearance of the disease.

There was no relationship between the age of gestation and the outcome of abortion in cattle. In addition there was no demonstrable evidence for the establishment of a persistent virus infection in the calves born to cows infected before 105 days of gestation.

Moderately and highly virulent strains of rinderpest virus were used to determine the time of onset of foetal infection which, with the moderately virulent strain, was first detected on the 17th day

after the onset of fever in the dam and on the 5th day following the onset of fever in the case of the highly virulent strain.

Evidence of transmission of infection by contact from aborting to susceptible cattle was equivocal.

Pregnant goats and rabbits inoculated with virulent strains of rinderpest virus aborted and unlike cattle, most of which aborted several weeks after recovery, abortions in goats and rabbits occurred during or soon after the acute phase of the disease. There was no virological or serological evidence of *in utero* infection.

ABBREVIATIONS

AGID	=	Agar gel immunodiffusion test
AVRI	=	Animal Virus Research Institute
BAPBS	=	Phosphate buffered saline containing 0.1 per cent bovine albumin
BK	=	Bovine kidney
BVD	=	Bovine virus diarrhoea
CD	=	Canine distemper
CPE	=	Cytopathic effects
EDTA	=	Ethylenediamine tetracetic acid
ES	=	Earle's salt solution
g	=	Gram
<u>g</u>	=	Centrifugal force
GK	=	Goat kidney
HBSS	=	Hanks's balanced salt solution
HEPES	=	N-2-hydroxyethyl-piperazine-N-2-ethane-sulphonic acid
ED	=	Effective dose
I.U.	=	International unit
IV	=	Intravenous
KAG	=	Kabete attenuated goat virus
kg	=	Kilogram
LN	=	Lymph node
MEM	=	Minimum essential medium Eagle
mm	=	Millimeters
nm	=	Nanometer

PBS	=	Complete phosphate buffered saline
PCV	=	Packed cell volume
p.i.	=	Post inoculation
PSM	=	Penicillin, Streptomycin, Mycostatin
RHS	=	Rinderpest hyperimmune serum
RV	=	Rinderpest virus
SC	=	Subcutaneous
SN	=	Serum neutralization
TCED	=	Tissue culture effective dose
ug	=	Microgram
μl	=	Microlitres
Vero	=	Cell line derived from African green monkey
v/v	=	Volume by volume
w/v	=	Weight by volume

INTRODUCTION

Rinderpest is a contagious and often fatal viral disease of cattle, buffaloes and other cloven-hoofed animals which up to the present day continues to cause havoc in large cattle populations in Africa and Asia. Although a considerable mass of information on the nature of the causative virus, and the pathogenesis and immunogenesis of the disease exists, information on the epidemiology of the disease is incomplete; in particular, the behaviour of the virus in pregnant animals has been ignored and its significance has not been assessed.

A few reports incriminate rinderpest virus in cattle and goat abortions. Early veterinary textbooks for example, Henning (1956) mention only briefly abortion as a clinical sign of rinderpest whereas recent texts such as Blood, Henderson and Radostits (1979), Fenner, Bachmann, Gibbs, Murphy, Studdert and White (1987) and Blood and Radostits (1989) do not. Reports on the recovery of virus from tissues and fluids of aborted foetuses following such infections are either contradictory or completely lacking. Similarly controversy surrounds the question of when rinderpest virus is eliminated from the body of an infected animal. The general consensus is that the virus disappears within three weeks of infection. However, there are reports of detection of virus in aborting and non-aborting cattle one to five months after recovery from the disease.

The objective of this study was to determine the effects of different strains of rinderpest virus in pregnant cattle, goats and rabbits.

CHAPTER ONE

REVIEW OF THE LITERATURE

Rinderpest has been recognised and feared as one of the most devastating clinical diseases of cattle since the 4th century when it caused a great pandemic in Europe (Barton, 1956). Thereafter the disease regularly swept through Europe from Asia often in the wake of the numerous military operations that ravaged the two continents (Gamgee, 1866). It is estimated that in the 18th century alone about 200 million cattle in Europe perished from the disease (Curasson, 1932). The need to control rinderpest undoubtedly became an important factor at this time and was the central core to the establishment of the first veterinary school in the world in Lyons in 1762. Similarly the first veterinary schools in Africa and Asia were established in Egypt in 1827 and India in 1872 primarily to produce trained personnel to help in the prevention of the then rampant rinderpest epidemics in these countries (Morcos, 1953; Ware, 1961). Modern veterinary science therefore owes it existence to rinderpest as are several international veterinary bodies like the present day World Veterinary Congress, the Office International des Epizooties in Paris and the Inter-African Bureau for Animal Resources in Africa. All were specifically initiated to deal with rinderpest.

Nevertheless rinderpest still remains one of the most important diseases of livestock to-day and continues to receive considerable scientific attention. Several workers have produced comprehensive reviews and described in excellent detail the history of the disease, the biological, physicochemical and antigenic features of the causative virus, and the clinical signs, the pathology and immunity of the disease (Ramazzini, 1711; Curasson, 1932; Henning, 1956; Plowright, 1968; Scott, 1985). A review of the effects of the disease on wild game in Africa and the influence on wildlife populations of its control in domestic stock was recently presented by Plowright (1982).

Layard (1757) was one of the earliest workers to write a classic description of rinderpest and was probably the first to associate bovine abortion with rinderpest infection. About one hundred and fifty years later, two French workers, Aldige (1918) in West Africa and Jacotot (1931) in Indochina recorded abortion as a sequel to natural and experimental infection of cattle with rinderpest virus respectively. Inexplicably, no follow-up studies to these observations have been made.

Aetiology

The establishment of the causal agent of rinderpest as a virus was made in 1902 by Nicholle and Adil Bey following their successful infection of cattle with bacteria-free filtrates of peritoneal fluids collected from clinical cases of rinderpest. Later numerous recommendations were made placing rinderpest virus under various classifications (Plowright, 1968). To-day however, rinderpest virus is included together with the viruses of human measles and canine distemper (CD) in the *Morbillivirus* genus of the *Paramyxoviridae* family (Kingsbury, Bratt and Choppin, 1978).

The grouping of the three viruses together arose from Polding and Simpson's (1957) epidemiological observation in Kabete, Kenya, that a dog population which was constantly fed meat from rinderpest-infected goats remained free from canine distemper, an observation which was later supported by the demonstration that rinderpest virus protected puppies against challenge with distemper virus (Polding, Simpson and Scott, 1959). At about the same time, Adams and Imagawa (1957) demonstrated an immunological relationship between CD and measles virus. Five years later, Plowright (1962a) showed a serological relationship between rinderpest and measles by demonstrating antibodies to rinderpest virus in the sera of children with measles and antibodies to measles virus in the sera of cattle convalescing from rinderpest. The Morbillivirus genus now includes the viruses of peste des petits ruminants (PPR) which causes epidemics of rinderpest-like disease in West African sheep and goats (Gibbs, Taylor, Lawman and Bryant, 1979). Other probable members of the Morbilli virus genus include marmoset paramyxovirus (Fraser, Chalifoux, Sehgal, Hunt and King, 1978), the paramyxovirus designated PMV 107 originally isolated from Germany cattle with encephalitis (Bachmann, Huppe, Muller, Mahnel and Meullen, 1979), the Hh1 virus from sick and healthy hedgehogs in England (Vizoso and Thomas, 1981) and the phocine distemper virus isolated from seals in the North Sea (Liess, Frey and Zaghawa, 1989).

Rinderpest virus is now considered to be the archetype of all these viruses (Norrby, Sheshberadaran, McCullough, Carpenter and Orvel, 1985) and although they are serologically related, they tend to be host specific except the viruses of rinderpest and PPR which both infect sheep, goats (Scott, 1955; Ali, 1984) and some wild ungulates (Scott, 1964; Furley, Taylor and Obi, 1987).

All members of the Morbillivirus genus are characterized by an antigenic relationship and possession of an RNA genome. Measles and rinderpest viruses possess an envelope containing haemagglutinin but not neuraminidase (Liess, 1964). Rinderpest virus particles as seen under an electron microscope are helically enveloped usually in spherical but sometimes in filamentous forms measuring 100-200 nm in diameter and 1μm in length respectively (Plowright, Cruickshank and Waterson, 1962).

Rinderpest virus is susceptible to various physical and chemical reagents. The virus survives best at low and high relative humidity but is rapidly destroyed at 50-60 per cent relative humidity (Hyslop, 1979). Light enhances the inactivation of the virus (Theiler, 1897). Virus inactivation occurs at temperatures above 37^{o}C (Scott, 1959a) but lyophilized virus is stable at low temperatures in the dark (Plowright, Rampton, Taylor and Herniman, 1970).

The optimum hydrogen ion concentration (pH) for the survival of rinderpest virus is 6.5-7.0 (Maurer, 1946; Liess and Plowright, 1963). Infectivity of rinderpest virus is generally destroyed when subjected to various chemicals especially lipid solvents (Plowright, 1962a; Scott, 1964; Plowright, Herniman and Rampton, 1971).

Transmission and pathogenesis

The principal mode of spread of rinderpest virus is by direct contact exposure of susceptible animals to infective aerosols generated by sick animals (Scott, 1957). It is considered that for effective transmission of infection to occur, the distance between the sick and susceptible animals has to be less than 2 meters and the contact has to last several hours (Idnani, 1944).

With regard to the host range, Rӧll (1860) wrote that rinderpest was a disease of cattle and buffaloes only but soon after, Bleisweis (1863) observed high losses in sheep and goats in Europe due to the disease. While no authenticated infections of pigs have been observed in Europe, Penning (1909) considered that pigs played a vital role in maintaining and propagating the disease in Southeast Asia. In East Africa rinderpest was present in the vast wild game populations before 1930 (Plowright, 1963a).

Different strains of rinderpest virus vary in their virulence for particular hosts and laboratory manipulations may alter some virus characteristics. The ease with which strains of rinderpest virus spread and infect animals also varies considerably within and between species. Some spread slowly in one species only while others spread very quickly between several species. Virulent and attenuated laboratory strains that do not spread by contact are also characterized by the non-development of mouth lesions, for example, the classic virulent Kabete "O "(MacOwan, 1956) and the tissue culture-attenuated vaccine strain (Plowright and Ferris, 1962). On the other hand, field strains including some that are avirulent for cattle produce mouth lesions and readily spread by contact; examples are the Ol Balbal and the RBT/1 strains (Robson, Arnold, Plowright and Scott, 1959; Plowright, 1963b). These observations led Plowright and Ferris (1959b) to suggest that the ability of rinderpest virus to spread by contact was linked to the development of oral lesions.

Cattle can be infected experimentally by any parenteral route of inoculation (Plowright, 1968). According to Hornby (1926) rectal deposition of the virus was not successful while varying results were observed following vaginal deposition (Todd and White, 1914). Taylor, Plowright, Pillinger, Rampton and Staple (1965) exposed healthy cattle to infected cattle for 24 hours and found virus localization in the pharyngeal, mandibular, bronchial and costo-cervical lymph nodes and also in the palatal tonsils of the exposed cattle. From these observations they concluded that natural infections of cattle with rinderpest virus usually occurs following passage of the virus through the epithelium of the upper and lower respiratory tract and its primary replication in the tonsils and associated lymph nodes. Thereafter, the virus attached to mononuclear cells is disseminated through the blood to other lymphoid organs and the mucosae of the alimentary and respiratory tracts (Plowright, 1968). Dissemination and replication of the virus in the mucosa of the urogenital tract has not been examined in great detail although recovery of rinderpest virus from urine of experimentally infected cattle has been reported (Liess and Plowright, 1964). The origin of the virus in the urine has not been established as post-mortem examination of even fatal cases has failed to show evidence of nephritis (Liess and Plowright, 1964). Although virus replication takes place in the epithelium of the lower urinary tract, the possibility that direct virus filtration through the glomerular sieve takes place has not been ruled out. Jacotot (1931) and Curasson (1932) demonstrated rinderpest virus in aborted foetuses and vaginal tract secretions from pregnant cattle that had aborted after rinderpest attacks. The site of virus replication in the reproductive organs is not known. The significance of the presence of virus in aborted foetuses relative to the virus multiplication cycle in the dam has not been investigated.

The clinical course of rinderpest infection in cattle is divided into five phases. While Plowright (1964) identified the incubation period, the prodromal fever, a mucosal phase and the convalescent phase which he linked to identifiable levels of virus replication in the host, Scott (1981) inserted a fifth, diarrhoeic phase between the mucosal and convalescent phases on the basis of the identifiable onset of diarrhoea.

The incubation period following contact exposure is variable and ranges from 4 to 15 days depending on the strain of virus (Robson et al, 1959; Taylor et al, 1965) and the innate resistance of the host (Scott, 1964). Experimentally, the incubation period lasts 3 to 9 days but this again depends on the strain of virus, dosage, route of administration and the innate resistance of the host (Plowright, 1968). During the first 3 days following the onset of pyrexia high titres of virus are reached and maintained in the lymphoid tissues, bone marrow and the mucosae of the alimentary tract. Virus also begins to proliferate in the nasal mucosae, lungs and liver and depending on the strain of the virus, appears in the blood a day before the onset of fever. The prodromal phase starts with a sudden onset of fever often reaching a peak of 41.5°C two to three days later. Affected animals become either restless or depressed, they are anorexic and in dairy cows the milk yield falls. The muzzle becomes dry and cracked, the visible mucosae are congested and serous to seromucoid nasal and ocular discharges develop and progressively become more profuse and mucopurulent. There is acceleration of respiration and heart rate, suppression of rumination and constipation. Most animals begin to show mucosal lesions between the 2nd and 5th days following the onset of pyrexia (Plowright, 1964; Taylor et al, 1965).

During the erosive mucosal phase of the disease which lasts up to the 7th day of fever, high titres of virus are found in the blood and all major sites of proliferation. Virus excretion becomes detectable in ocular, nasal and oral secretions, milk, urine and faeces starting from the first day of viraemia to about the 14th day after the onset of illness (Plowright, 1968; Mushi and Wafula, 1984). During this phase of infection there develops mouth lesions. Foci of necrosis, superficial erosions and capillary haemorrhages appear in the mucosa of the mouth, especially on the gums, lips, lower surface of the tongue, cheeks and hard palate and also in the nose and genital tract. These pinhead greyish lesions coalesce, and their necrotic centres become readily abraded leaving irregular sharply demarcated shallow erosions with raw red floors. The oral lesions stimulate profuse salivation and a pungent, fetid breath. Respiration becomes painful and laboured. The visible mucosal lesions usually resolve 2 to 5 days after their appearance but occasionally persist up to the convalescent phase.

The amount of virus declines rapidly in the late diarrhoeic and early convalescent phases with the appearance of antibody which occurs from the 6th day of the disease. Diarrhoea appears from the 4th to the 7th days following the onset of pyrexia and is at first watery but later contains blood, mucus and strands of intestinal mucosa. In severe cases the diarrhoea results in rapid dehydration, weakness, prostration and finally a semi-comatose state. Death usually occurs from the 6th to the 12th days of pyrexia but this is occasionally delayed to the 3rd week. The mortality rate may be over 90 per cent in highly susceptible cattle exposed to highly virulent strains of the virus.

During the convalescent phase there is complete disappearance of virus in tissues. Tissues like the cephalic lymph nodes which show the first viral proliferation are also those in which the decline first begins while the alimentary tract mucosae, the respiratory tract tissues and lymphoepithelial structures like the tonsils retain virus longest but for not more than 16 days (Plowright, 1964). Diarrhoea may persist into the convalescent phase and complete recovery from a severe attack requires at least 4 to 5 weeks. Pregnant cows that survive the attack abort 3 to 12 weeks after the onset of illness (Jacotot, 1931). In addition, Jacotot (1931) demonstrated rinderpest virus in tissues of 2 out of 17 aborted foetuses and in one out of three vaginal discharges examined at the time of abortion.

Infection of pregnant animals

Layard (1757) included in his review of the rinderpest pandemic that affected England in the 18th century personal observations of abortion in infected cows. Nocard and Leclainche (1896) also mentioned that abortion was a constant complication in rinderpest but did not provide details.

While working in West Africa, Aldige (1918) reported abortions in cattle as being virtually constant in all forms of natural rinderpest except in peracute and benign cases. He observed in Dakar, Senegal many abortions in cattle that had contracted natural rinderpest but had recovered completely. Due to good care and management the Senegalese stockowners had successfully saved a high proportion of the affected animals but abortion occurred in several of them nearly 3 months after recovery when four and a half months pregnant.

Aldige's observations that natural rinderpest induced abortion in cattle were confirmed experimentally by Jacotot (1931) who inoculated pregnant cows with serum and virus simultaneously and reported that the technique frequently led to abortion. In one of his experiments he observed the expulsion of 17 foetuses between 21 and 86 days following infection of cows in mid to late gestation. He recorded, as evidence of virus presence in 2 of the 17 expelled foetuses, the transmission of infection from the blood of the foetuses to rinderpest susceptible calves. Abortion in these two cases had occurred 21 and 33 days after inoculation. He also demonstrated the presence of virulent virus in vaginal fluids obtained from a cow that had aborted 34 days after inoculation with serum and virus simultaneously. He therefore concluded that pregnant cattle infected with rinderpest virus faced potential abortion and were capable of transmitting and propagating the virus in their foetuses and reproductive tract following natural or simultaneous serum-virus inoculation.

In East Africa, Scott (1963) recorded abortion rates of approximately 18 per cent in cattle following vaccination with lapinized rinderpest virus. On the basis of this observation, he advised against vaccination of susceptible pregnant animals with lapinized rinderpest virus. In contrast Plowright and Ferris (1962) failed to observe clinical signs of illness in ten heavily pregnant high grade cows following parenteral inoculation with a cell-culture attenuated rinderpest virus vaccine. The abortive effects of rinderpest virus in cattle therefore probably depend on the virulence of the infecting virus strain and the gestational age at the time of infection.

Abortion has also been reported in goats undergoing rinderpest infection (Banerji and Mohan, 1935). The incidence of natural rinderpest in this species was rare until the 1930's when outbreaks became frequent in India allegedly because of the haphazard passages of the virus in goats in an attempt to produce goat adapted virus vaccine (Scott, 1964). The technique was first developed by Edwards (1927) at Mukteswar in India while attempting to improve the serum-virus simultaneous method of immunization by passaging virulent bovine virus serially in goats. In one study he inoculated virus directly into the foetal membranes in a pregnant goat and recovered rinderpest virus in the blood of the mother three days later. Soon afterwards, Banerji and Mohan (1935) also working at Mukteswar observed abortion in a goat one week following inoculation with a strain of the "goat-adapted" rinderpest virus. A pool of spleen and blood collected from the aborted foetus induced rinderpest when inoculated into two susceptible goats. They concluded that the placenta of goats and probably the placenta of ruminants in general will permit the passage of a minute pathogen such as the virus of rinderpest.

Several years later, Chawla and Sinha (1961) while at Izatnagar in India demonstrated the presence of rinderpest virus in foetal goat spleens obtained from pregnant dams that had 4 days previously been inoculated with either bovine or caprine strains of rinderpest virus. The demonstration of the presence of virus was achieved by inoculation of susceptible goats and hill bulls with the foetal spleen homogenates and observation of the development of clinical disease or resistance to challenge with virulent virus.

There is a long record of failures and limited successes of infection of the rabbit with rinderpest virus (Scott, 1964). The conflicting results that thus exist have been considered to indicate a wide variation in the susceptibility of the rabbit to bovine-derived strains of the virus. For example, Morcos (1931), Philippe (1939) and Scott (1959b) failed to infect rabbits with different strains of the virus. On the other hand Hornby (1926) and Jacotot (1932a,b) both lost their virus after 3 passages but Inoue (1934) and Cebe and Perrin (1935) successfully passaged both laboratory and field strains in the rabbit. In 1938, Nakamura, Wagatuma and Fukusho reported the successful passage and adaptation of rinderpest virus in rabbits and developed the Nakamura III lapinized vaccine strain in Korea. The tissue culture vaccines in current use in some parts of Asia stem from these preliminary investigations. Similarly Baker (1946) in the United States of America propagated rinderpest virus in rabbits but this strain appears to have been lost.

Variations within and between breeds of rabbits to infection with rinderpest virus have been reported (Mornet, Orue, Labouche and Mainguy, 1953, Brotherston and Brown, 1955) and there is fragmentary information which suggest that the age of the rabbit influences its response to rabbit-adapted virus (Haddow and Idnani, 1947). Brotherston (1951) and Simpson (1954) reported that pregnant rabbits were more resistant to rinderpest virus than non-pregnant animals but unfortunately they did not provide the data on which they based their views.

While investigating the possible transmission of rinderpest virus across the goat's placenta, Chawla and Sinha (1961) in addition inoculated a pregnant rabbit with the Nakamura III strain of rinderpest virus. Foetal spleens and livers, collected post mortem at the height of fever were homogenized and inoculated intravenously into 8 rinderpest susceptible rabbits. Although the latter failed to show significant thermal reactions to the inoculum they proved immune to challenge with virulent lapinized virus. From these observations they concluded that rinderpest virus was capable of transmitting across the rabbit placenta.

Reliable information on rinderpest infection in pregnant sheep is scanty. A report by the Edinburgh Cattle Plague Committee (Anon, 1866) set up to inquire into, among other things, the susceptibility of sheep to cattle plague records a number of naturally acquired clinical cases of the disease in sheep in which several pregnant ewes aborted. In one instance, an aborted foetus from one of the affected ewes had characteristic pathological lesions of rinderpest (Anon, 1866).

On the other hand, there is no report on the pathological response of the bovine, caprine and lapine foetuses to infection with rinderpest virus and, apart from limited qualitative data in cattle

(Jacotot, 1931) there is no information on the quantitative proliferation of virus in the reproductive organs of infected pregnant animals.

Existence of carriers

There is a general consensus of opinion in veterinary circles that animals which recover from rinderpest infection do not become carriers or persistent excretors of virus. This view conflicts with a number of reports which claim to have isolated rinderpest virus from various tissues and secretions of cattle that had recovered, several weeks previously, from acute clinical rinderpest.

Curasson (1932) for example, reported the isolation of rinderpest virus from faeces, milk, suspensions of alimentary tract mucosae and vaginal discharges from cattle that had recovered 30 days earlier from acute clinical rinderpest. Similarly, Datta and Rajagopalan (1932) reported to have recovered rinderpest virus from the spleen of a hill bull inoculated with rinderpest virus 76 days previously. Gibbs in 1933 isolated virus from pyloric ulcers in 4 out of 200 cattle slaughtered 6 months after recovery from rinderpest. More recent reports include the recovery of virus from tissues of pigs 36 days after experimental infection (Delay, Moulton and Stone, 1962) and from the nasal mucosae of a case of "chronic" rinderpest killed 45 days after the onset of illness (Anon, 1971).

The maintenance of rinderpest virus in endemic areas is believed to depend upon the existence of susceptible domestic livestock and possibly wildlife species but is not due to the presence of carriers (Plowright, 1986). If claims of the existence of carriers were to be firmly established this would have far reaching implications with regard to the epidemiology and control of rinderpest. Japanese workers have recently established a Vero cell line persistently infected with rinderpest virus (Kobune, Yamanouchi, Nagashima and Shishido, 1981) while at Pirbright in the United Kingdom, an integration of rinderpest viral nucleic acid into host cell genome of rinderpest vaccinated cattle has recently been alleged.

The latter finding might explain the solid life-long immunity that occurs in cattle following recovery from natural infection or vaccination with attenuated rinderpest virus vaccine, and would suggest occurrence of carriers following infection with rinderpest virus. The role of such virus carriers as sources of infection to other cattle has not been shown and there is doubt that they represent a potential mechanism for the maintenance of infection.

The recent resurgence of rinderpest in many countries in the world (Plowright, 1986) has led to a global call for the establishment of international rinderpest control campaigns and hopefully the final eradication of the disease. The success of such an undertaking demands among other requirements that the vaccinal virus strain be safe both to the inoculated animal and other susceptible in-contact individuals including developing foetuses and they should not induce a virus carrier state.

Recent surveillance and computer simulation studies of the behaviour of rinderpest virus in cattle populations have suggested that virulent strains of the virus are more likely to lead to epidemic situations and that mild strains tend to lead to endemic rinderpest (Rossiter and James, 1989; Rossiter and Wamwayi, 1989). However, more important is the susceptibility of the host population to the virus that determines the occurrence of epidemic or endemic rinderpest, the former being easy to recognize and control and the latter being difficult to diagnose and eradicate. Rinderpest simulation studies and eradication strategies therefore require a proper understanding of the biological behaviour of the virus, its host and their interaction. There is no information on the behaviour of different strains of rinderpest virus in individual pregnant cattle and herd populations. Such data is required.

CHAPTER TWO

RESPONSES OF PREGNANT CATTLE TO INFECTION WITH DIFFERENT STRAINS OF RINDERPEST VIRUS

INTRODUCTION

Although comments associating rinderpest infection and abortion in cattle were first made by Layard in 1757, it was not until one and a half centuries later that the observation was first put to a controlled experimental test by Jacotot (1931). Even then no investigations were made to provide detailed data on the virological and immunological responses of the bovine foetus to laboratory and field strains of the virus.

Different strains of rinderpest virus vary considerably in their virulence for particular hosts and experimental passage in animals and cell cultures may alter strain characteristics. For example, Edwards (1927) noted that adaptation of an ox strain of rinderpest virus to goats fortuitously resulted in an increased virulence for goats and attenuation for cattle. His opinion was later confirmed by well-defined experiments by Saunders and Ayyar (1936) who re-investigated the effect of prolonged goat passage on the cattle virulence of an Indian strain of rinderpest virus and found evidence of attentuation after 80 passages. In Kenya, Daubney (1948, 1949) summarized the results of passage of the Kabete "O" strain in goats and recorded an induction of 100 per cent cattle mortality at the 50th goat passage level and a reduction in mortality to approximately 2 per cent after 250 goat passages.

The Nakamura III lapinized strain of rinderpest virus, developed by Nakamura and others (1938) is highly virulent for the rabbit through which it has been serially passaged hundreds of times but is attenuated for cattle. Plowright and Ferris (1959b) on the other hand found that passage of the Kabete "O" strain in cell cultures up to 10 times produced a highly virulent strain for cattle. From the 16th passage onwards, increased attenuation in the virus progressively occurred to a level where the 90th culture-passaged virus is now widely used as an attenuated vaccine for cattle (Scott, 1985).

Naturally occurring field strains of rinderpest virus also differ in their virulence for cattle. Robson and others (1959) isolated rinderpest virus from a sick eland which even after 9 passages in cattle was only mildly virulent for the latter. The eland strain thus resembled a naturally attenuated field strain previously isolated from cattle in Tanzania (Lowe, Wilde, Lee and Stuchbery, 1947) and 7 other non-lethal strains later isolated from cattle in East Africa (Plowright, 1963b). In contrast the RGK/1 strain of rinderpest virus isolated from a sick giraffe in Northern Kenya (Leiss & Plowright, 1964) and the strain responsible for the epizootic which occurred in the northern districts of Kenya in 1960 killing buffalo and other wild game (MacOwan, 1961) also proved highly virulent for cattle.

In the present studies groups of pregnant cows were inoculated with a moderately attenuated caprinized vaccine strain, a mildly virulent laboratory strain, a moderately virulent field virus isolate or a highly virulent laboratory strain of rinderpest virus. Infected animals were clinically examined every day paying particular attention to signs of abortion. Thereafter evidence of _in utero_ infection of their foetuses was also sought.

MATERIALS AND METHODS

Cell cultures

Bovine kidney cells

Primary bovine kidney (BK) cell cultures were prepared by conventional methods of trypsinization (Plowright and Ferris, 1959a). Kidneys were asceptically removed from bovine calves aged 7-10 days in the post-mortem room. The capsules were removed and the cortices sliced off the kidneys and washed once with phosphate buffered saline (PBS) containing antibiotics. The cortices were again washed with Hanks's balanced salt solution (HBSS) before trypsinizing in 0.25 per cent trypsin[1] in deionised water (w/v) for 1.5 hours at 37°C. The trysinized cell suspension was sedimented by centrifugation at 400 g for 5 minutes and the packed cell volume (PCV) determined. The cells were then diluted 1/120 final PCV in growth medium, seeded at 25 ml in 300 ml culture bottles then incubated at 37°C. The growth medium consisted of HBSS supplemented with 0.5 per cent lactalbumin hydrolysate and 10 per cent ox serum. Growth medium was changed after the 3rd day to Earle's maintenance (ES) medium supplemented with 2 per cent ox serum. Cultures were usually confluent by the 6th or 7th day.

Subculture

Secondary BK cell cultures were prepared by subculturing of

[1] Difco Laboratories Ltd., Detroit, U.S.A.

primary monolayers. Confluent monolayers were washed twice with warm PBS then 5 ml of warm versene/trypsin (0.02% EDTA plus 0.1% trypsin) were added to a 300 ml culture bottle and incubated at 37°C for 5 to 10 minutes. The detached cells were deposited by centrifugation at 400 g for 5 minutes and resuspended in Minimum Essential Medium (MEM) Eagle[1] as growth medium. The cells were then counted in a haemocytometer chamber, diluted to give 10^5 cells per ml and seeded in Pyrex-glass culture test-tubes (16 x 150 mm). The tubes were then closed by red rubber bungs[2] and incubated at 37°C for 4 days sloped in a stationary position. The medium was then replaced with ES medium supplemented with 2 per cent ox serum and the tubes transferred to a roller drum for re-incubation.

Bovine testis cells

Primary and secondary bovine testis (BT) cells were prepared by a method identical to that described for BK cells.

Virus strains

The Kabete attenuated goat (KAG) strain of caprinized rinderpest virus was kindly supplied by Dr. E. Anderson, Animal Virus Research Institute (AVRI), Pirbright, United Kingdom. It consisted of a freeze-dried suspension of infected goat spleen which had undergone more than 600 goat passages. The virus induces only transient fever in cattle (Daubney, 1947) and is here referred to as a moderately attentuated strain (Table 2.1). It was used to infect animals after a single goat passage at Muguga.

[1] Wellcome Ltd.
[2] ESCO (Rubber) Ltd., Feltham, U.K.

The RBT/1 strain was also kindly supplied by Dr. Anderson as a freeze-dried infected cattle spleen. The virus, originally isolated in Arusha Tanzania from the blood of a sick cow induces only fever and mouth lesions in cattle (Plowright, 1963b) and is here referred to as a mildly virulent strain (Table 2.1). It was used to infect cattle after one passage in BK cell cultures at Muguga. The previous passage history of the virus was not known.

In August 1988, a rinderpest outbreak occurred in cattle in Kenya's central districts of Kiambu, Kajiado and Nairobi (see Chapter Five). A cytopathic virus, here designated RBK/Kiambu/88/1 (RBKK) was recovered in BK cells from the blood of a sick cow found in Kiambu district. The virus induced fever, mouth lesions and diarrhoea after inoculation into susceptible cattle (Wamwayi, Kariuki, Wafula, Rossiter, Mbuthia and Macharia, 1989) and is herein referred to as moderately virulent (Table 2.1). It was identified as rinderpest virus following neutralization with rinderpest hyperimmune serum. The virus was used as infected whole blood in EDTA or as infected cell culture fluids after 2 passages in BK cells.

The Kabete "O" (RBKO) strain of rinderpest virus had previously been maintained for over 40 years by subcutaneous (SC) inoculation of cattle with infected spleen suspension (MacOwan, 1956). Its behaviour was regular, with inoculated cattle showing high temperature reactions by the 2nd or 3rd day. It did not produce mouth lesions neither did it spread readily by contact to susceptible cattle (Brotherston, 1951; Plowright, 1952). The virus was subsequently passaged 5 times in BK cell cultures whereupon an enhancement of virulence occurred manifested by an increased mortality rate, a decreased time to death, development of mouth lesions and transmission by contact (Plowright and Ferris, 1959b). The virus, hereafter, referred to as highly virulent, (Table 2.1), was used as a 20 per cent infected cattle spleen suspension after the 5th passage in BK cells and a single passage in cattle.

From the 16th passage level onwards the virus became increasingly attenuated for cattle (Plowright and Ferris, 1959b). This attenuated strain was used in serological tests after the 90th BK culture passage.

The NADL strain of bovine virus diarrhoea (BVD) virus also supplied by Dr. Anderson as a freeze-dried cell culture-passaged virus was used after 6 passages in BT cells at Muguga. The virus was originally isolated in the United States of America from a spleen of a calf with naturally occurring BVD (Gutekunst and Malmquist, 1963).

Rinderpest hyperimmune serum

Rinderpest hyperimmune serum (RHS) was raised in rabbits using method II of Scott (1967). Briefly, 4-month-old New Zealand White rabbits were passively immunized by intravenous injection of 2 ml rinderpest hyperimmune serum per kilogram (kg) body weight. The rabbits were then intravenously injected within 24 hours with 1.0 ml of a 2 per cent suspension of a spleen from a rabbit infected with the Nakamura III strain of rinderpest virus (Nakamura et al, 1938).

The animals were further injected with 1.0 ml, 2.0 ml, and 4.0 ml of the same virus preparation on the 7th, 11th and 15th days post immunization respectively and test bled on day 23. Normal rabbit serum was collected from healthy uninfected rabbits over 4 months of age.

Cattle

Three-to-five-year-old *Bos taurus*-*Bos indicus* crossbreed (grade) or *Bos indicus* (Zebu) cows were used when 12-37 weeks pregnant. In addition one-to-two-year-old grade steers were used in contact transmission studies. All animals were screened for neutralizing antibodies to rinderpest virus prior to use. The cattle were housed in disease-secure accommodation and provided with hay and water *ad libitum*.

Cattle inoculation

Infection with the KAG virus strain

Four adult Zebu cows between 32 and 37 weeks pregnant were each inoculated SC with 2 ml of a 20 per cent suspension of spleen harvested on the 2nd day of fever from a goat infected with KAG virus.

Infection with the RBT/1 virus strain

Four Zebu cows, 3 to 4 years old and between 23 and 36 weeks pregnant were each injected SC with 2 ml cell culture-passaged RBT/1 virus suspension containing $10^{2.7}$ median cell culture effective dose ($TCED_{50}$) per ml of suspension.

Infection with the RBKK virus isolate

Two Zebu cows, X3 and X19 each 4 years old and about 12 and 23 weeks pregnant were inoculated intraveneously (IV) with 20 ml whole blood in EDTA from a sick cow infected naturally with rinderpest virus. The virus titre in the inoculum was estimated to be $10^{1.4}$ $TCED_{50}$ per ml of blood.

Two more adult Zebu cows, X93 and X94, about 17 and 23 weeks pregnant were kept in close contact with the two IV-inoculated cattle from the day of inoculation.

Two cows X106 and X110, also more than 3 years old and 12 and 23 weeks pregnant were injected SC with 2 ml of a 20 per cent (w/v) spleen suspension harvested on the 3rd day of fever from a steer previously infected as cows X3 and X19 above. The virus content in the suspension was estimated in BK cell cultures to be $10^{1.8}$ $TCED_{50}$ per g of spleen.

Infection with RBKO virus strain

Four grade cattle aged 3-5 years and 28-34 weeks pregnant were each inoculated SC, with 2 ml spleen suspension containing an estimated infectivity titre of $10^{5.2}$ median cattle effective dose (ED_{50}) of the highly virulent RBKO virus. The virus titre in the spleen inoculum was determined by titration in susceptible grade steers.

Detailed clinical examinations were carried out daily and continued until two weeks after recovery. Thereafter, the animals were observed daily until the time of abortion or normal delivery when further detailed examinations were resumed and continued for another 2 weeks.

Assessment of clinical parameters

The incubation period was taken as the number of days between the time of inoculation and the onset of fever i.e. the day of inoculation was considered as day 0 and the day preceding the onset of fever as the last day of incubation.

Fevers were recorded as the number of days the animals were actually febrile i.e. had morning temperature reactions of greater than 39.5^{o}C for cattle and goats, and 40.0^{o}C for rabbits.

Days of clinical disease were recorded with the onset of fever as the baseline. Similarly, the onset of fever was taken as the baseline for virological assays. Statistical analyses were performed by parametric tests mentioned in the relevant sections.

Collection and processing of samples

Maternal, aborted foetal and newborn calf blood in EDTA and without anticoagulant were collected daily and soon after parturition or abortion in vacutainer tubes from either the jugular vein, umbilical cord or the heart.

Ocular, nasal and vaginal secretions were collected on sterile absorbent cotton swabs by inserting the swabs into the conjunctival sac, nostril or posterior vaginal tract respectively and leaving them in position until they became wet. The swabs were then immersed into 3 ml BAPBS supplemented with 400 iu/ml penicillin, 400 ug/ml streptomycin and 200 iu/ml mycostatin (PSM). Foetal thymus, prescapular and mesenteric lymph nodes, lung, liver, spleen, kidney, abomasum, cotyledons, membranes and fluids were collected soon after abortion into sterile universal bottles.

Leucocyte fractions were separated from the EDTA blood by centrifugation at 400 g for 10 minutes at 4°C. The resulting buffy coat was washed twice in PBS then re-suspended in PBS to the original volume. The swab extracts and foetal fluids were agitated and ten-fold dilutions prepared in BAPBS. Solid tissues were homogenized using sterile mortars and pestles as 10 per cent tissue suspensions in PBS. Virus titres were calculated by the method of Spearman-Kärber (Lennette and Schmidt, 1964). Where the lowest sample dilution failed to give 100 per cent viral cytopathic effects (CPE) virus titres were calculated by transforming the negative logarithm of the dilution to one log higher then back-transforming it after calculating the titre (Appendix 1).

Inoculation of cell cultures

Ten-fold dilutions of buffy coat and tissue suspensions were prepared in BAPBS. Five-to-7-day-old confluent monolayers of secondary BK cells in culture tubes were inoculated with either 0.2 or 1.0 ml per tube of the sample dilutions using 5 culture tubes per dilution. Inoculated tubes were incubated in roller drums at 37°C overnight. The inoculum was then decanted and the cultures washed twice with warm PBS. ES medium containing 2 per cent ox serum was added and the tubes further incubated at 37°C. The cultures were examined for the development of CPE for 21 days, the medium being changed every 3 or 4 days.

Agar gel immunodiffusion test

The agar gel immunodiffusion (AGID) test was run by allowing tissue exudates and homogenates of aborted foetal membranes, thymus, lung, spleen, liver, kidney, abomasum, cotyledons, prescapular and mesenteric lymph nodes to diffuse through the agar against RHS. The test was essentially that described by Scott and Brown (1961) and modified by Forman, Rowe and Taylor (1983).

The known positive rinderpest control antigen was prepared from rinderpest virus infected bovine kidney cells which remained after the routine production of tissue culture rinderpest virus vaccine (Plowright, 1962b). Culture overlay medium was collected when the infected cell sheet showed more than 80 per cent CPE and the cells mechanically removed from the glass wall using a rubber policeman. The cells were washed twice in PBS, resuspended in PBS to 1 per cent of their original tissue culture fluid volume and ultrasonicated on ice at 20 kHz for 2 cycles of 10 seconds. The disrupted cell suspension was centrifuged at 400 g for 5 minutes and the supernatant stored in 0.2 ml aliquots at -20°C until used as antigen.

Negative control antigen was prepared from uninfected BK cells in a manner similar to that for positive control antigen. The test was performed in 1 per cent Agar Noble[1] in deionised water plus 0.02 per cent sodium azide. Five ml of gel were dispensed into 4.5 cm diameter plastic petri dishes and a cutter designed to simultaneously cut wells 3 mm in diameter and 2 mm apart was used to stamp the agar gels. Volumes of 12.5 µl of antisera and antigen were used in control and test wells and final readings taken after 24 hours of incubation at 37°C in a humidified container. The test was considered positive if the precipitation lines between test material and positive control antigen merged together.

[1] Oxoid Ltd., Hants, U.K.

Test for antibodies to rinderpest virus

Tests for the presence of neutralizing antibodies to rinderpest virus in animals were carried out in microtitre plates as described by Rossiter and Jessett (1982). Briefly, sera or aborted foetal tissue exudates were heat-inactivated with constant shaking at $56^{o}C$ for 30 minutes in a waterbath. A two-fold dilution series of each sample was then made in BAPBS using microtitre plates and 0.025 ml microdilutor loops. Three wells were used for each dilution of serum and the lowest dilution of serum tested was 1/2. Rinderpest virus suspension containing an estimated $10^{3.0}TCED_{50}$ per ml was added in 25 µl volumes to each well. Following incubation of the virus-serum mixture at $37^{o}C$ for 1 hour, 0.15 ml of BK cell suspension containing 2×10^{5} cells per ml of MEM supplemented with antibiotics, HEPES buffer and 10 per cent ox serum was added to each well and the plates sealed with adhesive tape.

The controls in every test consisted of a positive and negative serum, uninfected cells and a virus titration. Plates were incubated at $37^{o}C$ in a humidified chamber and examined every 3 days for the presence of viral CPE.

The median serum neutralizing (SN) antibody titres were calculated by the method of Spearman-Kärber (Lennette and Schmidt, 1964).

Test for antibodies to bovine virus diarrhoea virus (BVDV)

Cattle sera were tested in microtitre serum neutralization test against $10^{2}TCED_{50}$ per ml of the prototype NADL strain of BVD virus using BT cells (Rossi and Kiesel, 1971). The lowest serum dilution tested was 1/2 and 3 wells were used for each dilution.

Test for antibodies to Brucella abortus

Cattle sera were examined for the presence of antibodies to Brucella abortus using the slide agglutination test as described by Alton and Jones (1963). Briefly equal volumes of standardized antigen and test sera were placed side by side on the test plate and thoroughly mixed using applicator sticks and then shaken for 4 minutes on a rocking machine. Results were read by checking for agglutination using a good source of light. Control positive and negative sera were included in each test.

RESULTS

Cattle infected with the KAG virus strain

Clinical signs

One cow developed fever of 40.0^{o}C on the 4th day after inoculation with the KAG virus strain and two had an incubation period of 4 days. One cow did not develop fever (Table 2.2). The duration of fever was 2 to 4 days. Slight lachrymation developed in the cows that developed pyrexia from the day of onset of fever and lasted 2 days. Diarrhoea and mouth lesions were not observed and abortion did not occur. The four animals delivered normal calves at term i.e. between 2 and 8 weeks after virus inoculation (Table 2.2).

Virus isolation

No virus was recovered in BK cells from either the EDTA blood or nasal, ocular and vaginal secretions from the four animals. Likewise virus was not demonstrated in the blood, nasal, ocular and vaginal secretions obtained from the calves and their dams soon after delivery.

Serology

Moderate amounts of virus neutralizing antibodies were demonstrated in the KAG-inoculated cattle on the 7th day after inoculation and had reached titres of 3.2 to 3.8 $\log_{10}SN_{50}$ by the 2nd week of inoculation (Table 2.3). No antibodies were detected in pre-colostral sera from the four calves but antibody titres up to 3.6 $\log_{10}SN_{50}$ had been acquired by the 2nd day of birth (Table 2.4).

Cattle infected with RBT/1 virus

Clinical signs

The four cows inoculated with the RBT/1 strain developed a rise in temperature greater than 39.5^{o}C on the 4th day after inoculation and the duration of pyrexia was 4 days in one cow and 5 days in three cows (Table 2.5). Peak pyrexia (40.0^{o}C) was attained in two cows on the 3rd day of fever, in one cow on the 4th day and on the 5th day in the last cow.

Mild ocular and nasal congestion with slight serous discharges were observed in three cows, one on the 2nd and two on the 3rd days of fever but were absent by the 4th day. Diarrhoea did not develop in these animals.

Necrosis and erosions on the lips, gums, lower surface of the tongue, hard palate and papillae tips were first observed in two cows on the 4th day of fever while a third cow had the lesions on the 5th day of pyrexia. The lesions were present for 2 to 3 days. The lesions were preceded by 1 or 2 days of generalized hyperaemia of the mucosae and were characterized by raised white foci of necrosis or small reddened erosions. The lesions however did not develop into extensive confluent necrosis in these animals and one cow (A14) completely failed to develop oral lesions.

Two cows (A13 and A20) produced full-term normal calves on the 13th and 103rd days following clinical recovery[1] from the disease i.e. 24 and 114 days after inoculation respectively. The other cows (A14 and A18) aborted 8-month-old dead foetuses on days 41 and 29 after recovery from clinical rinderpest respectively (Table 2.6) i.e. on the 52nd and 40th days of inoculation respectively. Abortions were preceded by straining and a slight clear mucoid vaginal discharge for 2 to 5 days. One cow, A14 retained the afterbirth for 3 days which was easily extracted manually. No cow died from infection with the RBT/1 strain and all animals showed complete clinical recovery 1-2 days after the remission of fever.

Virus isolation

Virus was first detected in the blood of two cows on the day of

[1] The first day of recovery was taken as the day following the disappearance of clinical signs of infection i.e. fever, mouth lesions and diarrhoea.

onset of fever but all the animals were viraemic on the 1st day of fever (Table 2.7 and Figure 2.1). The time from inoculation to detection of virus in the blood was thus 4 to 5 days in the four cows. The median peak titre for viraemia was $10^{2.5}$TCED$_{50}$ per ml attained on the 4th day after the onset of fever. The highest blood titre of $10^{2.6}$TCED$_{50}$ was detected in cows A18 and A20 on the 4th day of fever (Figure 2.1). Viraemia in the four cows lasted 6 to 8 days. Virus was recovered from vaginal secretions collected from cow A18 on the 2nd and 3rd days after the onset of fever. Virus was not detected in nasal, ocular and oral swabs taken from the four cows during the course of the disease. Aborted foetal blood, tissues and fluids did not yield virus in BK cultures.

Antigen detection

Rinderpest virus antigens were not detected by AGID test in aborted foetal spleen, thymus, mesenteric lymph nodes, lung, kidney, abomasum and cotyledons obtained from the two aborted foetuses.

Serology

Moderate levels of virus neutralizing antibody to rinderpest virus had developed in the four cows by the 7th day of inoculation (Table 2.8). Antibody titres rose to high levels by the 2nd week of inoculation. No antibodies to BVD virus and B. abortus were detected in the four cattle sera when tested at the time of delivery or abortion.

Neutralizing antibodies were not detected in pre-sucking serum of the calf born to cow A13 although the dam had a serum antibody titre of 2.9 $\log_{10}$SN$_{50}$ at the time of delivery (Table 2.6). The calf however had an antibody titre of 2.2 $\log_{10}SN_{50}$ when tested 7 days after birth.

A serum neutralizing antibody titre of 2.6 $\log_{10}SN_{50}$ was demonstrated in the pre-sucking serum of the calf born to cow A20. The dam's serum antibody level at the time of delivery was 2.9 $\log_{10}SN_{50}$ (Table 2.6).

Antibodies to rinderpest virus were not detected in the sera and tissue exudates from mesenteric lymph nodes, lung, kidney, abomasum and cotyledons obtained from the two aborted foetuses.

Post-mortem changes

None of the cows infected with the RBT/1 virus strain died from the infection. Foetuses aborted by cows A14 and A18 appeared grossly normal. There were however a few necrotic foci in the foetal cotyledons.

Cattle infected with the RBKK virus isolate

Clinical signs

The two cows inoculated intravenously with the EDTA blood from the sick cow in Kiambu developed fever of 40.0^{o}C on the 5th and 6th days of inoculation and the duration of fever was 12 and 5 days respectively (Table 2.9). Pyrexia was in both cases accompanied by hyperaemia of the visible mucous membranes and a serous to seromucoid nasal and ocular discharge which lasted 3 days. Mouth lesions in the form of focal necrosis and erosions of the epithelia were first observed on the 10th and 5th days of pyrexia. The lesions healed after 3 days. A slight watery diarrhoea developed in cow X3 on the 1st day of fever and remained so until the 5th day when it became profuse and bloody. After one day the diarrhoea stopped. Diarrhoea did not occur in cow X19.

Both cows aborted 4-and-6-month-old foetuses on the 30th and 35th days respectively following clinical recovery. Cow X3 retained her placenta for 2 days before it was manually extracted (Figure 2.2).

The incubation periods in the two cows X93 and X94 infected by contact with the intravenously inoculated cattle were assumed to be 10 and 9 days respectively from the 1st day of fever in cow X3 (Table 2.9). This assumption was based on and later justified by data on virus secretion in which virus was not demonstrated in the tears of cow X3 until one day before the onset of fever (Table 2.11). The duration of fever in the two cows was 6 and 7 days respectively and the pattern of clinical signs was similar to that observed in cows X3 and X19 except that diarrhoea occurred in cow X93 on days 6 to 8 after the onset of fever. Cow X93 aborted a 5-month-old foetus on the 23rd day following clinical recovery from the disease while cow X94 aborted a 5-month-old foetus two days after the fever regressed.

The incubation periods in the subcutaneously inoculated cows, X106 and X110, were 6 and 4 days respectively (Table 2.9) and the durations of fever were 5 and 4 days. Oral necrosis and erosions were observed in both cows from the 4th to the 6th day after the onset of fever. Cow X110 aborted a 6-month-old foetus on the 30th day following clinical recovery from the disease. Cow X106 produced a normal calf at term i.e. 170 days after recovery from the disease. The 6 animals infected with the RBKK virus isolate generally showed clinical recovery from the disease 1-2 days after the remission of fever.

Virus isolation

Viraemia was detected in 2 animals one day before the onset of fever, in 3 animals on the day of onset of fever and in the last animal one day after the onset of fever. The duration of viraemia ranged from more than 3 to 7 days with a median of 6.5 days (Table 2.10). Median peak viraemia of $10^{1.5}TCED_{50}$ per ml of blood was attained on the 4th day of fever (Figure 2.3) with the highest blood titre of $10^{1.6}TCID_{50}$ per ml of blood detected in cow X93 on the 4th day of fever. The duration of viraemia in cow X110 was not established because of bacterial contamination of cell cultures.

Virus was first detected in the tears of cow X3 on the day preceding the onset of fever but all the animals were secreting virus through the tears on the 2nd day of fever (Table 2.11; Figures 2.4 and 2.5). Peak median secretion of virus in ocular discharges was $10^{1.9}TCED_{50}$ per swab and was attained on the 4th day of fever. Virus secretion in tears had stopped in all cows by the 8th day following the onset of fever.

The secretion of virus through the vaginal tract was first detected one day after the onset of pyrexia and was first recovered in one IV inoculated cow (Table 2.12; Figures 2.6 and 2.7). Peak median virus titre of $10^{1.5}TCED_{50}$ per swab was detected in the vaginal secretions on the 4th day of fever but progressively declined to undetectable level by the 8th day after the onset of fever.

Rinderpest virus was not recovered from any of the 6 cows after the 8th day following the onset of fever and neither was it isolated in BK cell cultures from the aborted foetal, newborn calf and maternal blood, secretions, fluids and tissues collected at the time of abortion or parturition and daily for 7 days thereafter.

Antigen detection

Rinderpest virus antigens were demonstrated in foetal thymus and mesenteric lymph nodes from foetuses expelled by cows X3, X19, X93 and X110. In addition virus antigens were detected in the spleen, lung and gum scrapings from the foetus expelled by cow X110 (Table 2.13). Rinderpest virus antigens were not demonstrated in foetal tissues and fluids from the foetus expelled by cow X94. It is worth noting that this cow aborted soon after recovery from the acute disease i.e. 2 days after the fever regressed. Cow X106 did not abort but produced a healthy, normal live calf 170 days following recovery from clinical disease. In both cases there was no evidence of *in utero* infection of the foetus.

Serology

The development of neutralizing antibodies in the 6 experimentally infected cows was typical (Plowright and Ferris, 1962) and was first observed in the parenterally inoculated cows on the 7th day of inoculation (Table 2.14). Peak neutralizing antibody levels were reached after the 2nd week in the parenterally inoculated cows but this was not attained until after the 3rd week of contact in animals infected by contact. Antibodies were not demonstrated in the sera and tissue exudates from the five aborted foetuses and in the pre-sucking serum of the calf born to cow X106 (Table 2.13).

Cattle sera collected at the time of abortion or normal delivery were devoid of antibodies to BVDV and B. abortus.

Post-mortem changes

All the 6 cows recovered from the disease. Four of the 5 aborted foetuses had generalized congestion and severe oedema in the liver, spleen and under the skin (Figure 2.8). The foetuses had in addition an accumulation of blood stained abdominal and thoracic fluids. There was necrosis of the hard palate and bucal papillae and erosions on the lips and tongue of the foetus expelled by cow X110 (Figure 2.9).

Cattle infected with RBKO virus

Clinical signs

The incubation period in the four grade cattle infected with the enhanced virulent RBKO strain of rinderpest virus was 3 days (Table 2.15). The duration of pyrexia was 2 days in two animals, 3 days in one animal and 5 days in the last animal. The three cows that had fever for 2 and 3 days died on the 3rd day of the onset of fever. The onset of fever was accompanied by hyperaemia and marked congestion of the ocular, nasal, oral and vaginal mucosae. A serous oculo-nasal discharge developed in all the cows from the day preceding the onset of fever and became profuse and mucopurulent 2-3 days later.The discharges ceased on the 7th day following the onset of fever in the cow that survived.

Oral lesions consisting of hyperaemia, necrosis and erosions on the lips, gums, papillae, hard palate and the lower surface of the tongue were first observed in two cows 2 days after the onset of fever and in the survivor on the 5th day. Similar lesions appeared simultaneously in the posterior vaginal tract of three animals and persisted until death or for 3 days in the survivor. One cow died without having developed mouth and vaginal lesions.

In the survivor, the lesions developed into extensive confluent necrosis of the mucosae but the epithelia rapidly regenerated and were complete within 3 days. Slight diarrhoea occurred in this animal on days 5 and 6 after the onset of fever. The cow recovered one day later. On the 12th day after recovery she started straining and developed an odourless straw-coloured discharge from the vagina. Four hours later the cow aborted a well preserved 38-week old dead foetus.

Virus isolation

Viraemia was first detected in the four cows one day before the onset of pyrexia and persisted until death or until the 5th day after the onset of fever in the survivor. The peak titre of viraemia in the survivor was $10^{2.6}TCED_{50}$ per ml of blood attained on the 2nd day of pyrexia (Table 2.16; Figure 2.10). Virus detection in the blood ceased after the 5th day following the onset of fever.

Rinderpest virus was detected in ocular and vaginal secretions from the 4 cows one day before and after the onset of fever respectively. Virus was detected in increasing amounts in these secretions until death or the 2nd day after the onset of fever in the survivor but declined steadily thereafter so that it was no longer detectable by the 5th day after the onset of fever. Virus was again detected in vaginal discharges on the day of abortion and again 24 hours later (Table 2.16; Figure 2.11).

Rinderpest virus was isolated from the spleen, liver, abomasum, lung, meconium, chorioallantoic membranes and cotyledons of the aborted foetus (Table 2.17). The titre of virus was highest in the cotyledons ($10^{2.6}TCED_{50}$ per g) but ranged between $10^{1.4}$ to $10^{1.8}TCED_{50}$ per g in other tissues. Virus was not recovered from tissues, membranes and fluids of the foetuses obtained from cows that died in the acute stage of the disease.

Antibody detection

Antibody development in the survivor cow was first detected on the 7th day after infection and reached a peak level by the 3rd week (Table 2.18). Neutralizing antibodies were not demonstrated in the sera from cows that died from the acute disease neither were they detected in any foetal serum. Antibodies to BVDV and B. abortus were not detected in this cow at the time of abortion.

Maternal post-mortem changes

Three out of the four cows died on the 3rd day following the onset of pyrexia. Post mortem examinations revealed severe erosive and haemorrhagic gastroenteritis, congestion and necrotic areas in the nasal mucosae and signs of mild interstitial pneumonia in the lungs. Congestion and necrosis of the placentomes were observed in two of the dead cows.

Foetal pathology

There was a straw-coloured exudate from the placentomes of one of the 3 cows that died and necrosis of the foetal cotyledons from the cow that aborted.

Relationship between gestational age and foetal response to infection of pregnant cattle with rinderpest virus

Of the 18 pregnant cows infected with the various strains of rinderpest virus, 3 died from infection with the RBKO virus and 15 survived. One cow, A20, infected at 23 weeks gestation produced a normal live calf at term with serological evidence of transplacental infection (Table 2.19a). Six cows infected between 12 and 37 weeks of gestation gave birth to normal live calves at term and there was no evidence of in utero foetal infection (Table 2.19a).

Five cows infected between 12 and 34 weeks of gestation aborted between 23 and 48 days following infection and there was virological and antigenic evidence of transplacental infection of the foetuses (Table 2.19b). Three cows infected between 23 and 27 weeks of pregnancy aborted between 17 and 52 days after infection. There was however no indication of in utero infection of the foetuses in these animals.

The results of Tables 2.19a and b therefore show that out of the 15 pregnant cows that recovered following infection between 12 and 37 weeks of gestation 8 (53 per cent) aborted between 17 and 52 days after infection. There was, however, no relationship between the gestational age at infection and the time to abortion ($r = 0.72$, $p>0.10$). In addition, infection of pregnant cattle with rinderpest virus did not influence the gestation period in animals that did not abort ($r = -0.99$, $p<0.001$) (Figure 2.12).

In 4 out of the 5 animals that aborted following exposure to the RBKK virus and in which there was evidence of virus infection in the foetus, the mean time and standard deviation to abortion was 44.5 ± 3.5 days (Figure 2.13). In contrast, the time to abortion in the one cow that survived infection with the RBKO virus was 23 days. This time was significantly shorter, lying outside the lower 99 per cent confidence limit of the time to abortion in the RBKK-infected animals i.e. 34.2 days. It is not clear therefore whether the difference is attributable to the gestational age of the foetus at the time of infection or to differences in the two strains of virus.

Comparative responses of pregnant cattle infected with different strains of rinderpest virus

The mean incubation periods in pregnant cattle parenterally infected with the various strains of rinderpest virus ranged from 3 to 6 days. Mouth lesions occurred in cattle inoculated with the mildly virulent RBT/1 virus, the moderately virulent RBKK virus and the highly virulent RBKO virus strain but not in animals infected with the moderately attenuated KAG virus strain (Table 2.20). Diarrhoea was observed in cattle infected with the RBKK and RBKO virus strains only. High mortality occurred in the RBKO virus infected cattle only. Abortion occurred in 50, 84 and 100 per cent of the cows that survived infection with RBT/1, RBKK and the RBKO viruses respectively. There was serological evidence of *in utero* foetal infection in one cow infected with the RBT/1 strain, antigenic evidence of foetal infection in four cows exposed to the RBKK virus and virological evidence of foetal infection in the cow that survived infection with the RBKO virus strain.

DISCUSSION

Experimental exposure of pregnant cattle to four strains of rinderpest virus of varying degrees of virulence resulted in clinical diseases of different severity.

The moderately attenuated KAG vaccine strain induced a low fever and slight lachrymation in pregnant cattle but no other signs of clinical disease. The animals were infected between 32 and 37 weeks pregnant and they all delivered normal live calves at term. There was no virological or serological evidence of transplacental infection in the calves. The clinical features observed in cattle infected with the KAG virus were therefore similar to those previously recorded (Daubney, 1947).

Mild clinical disease characterized by fever and mouth lesions developed in cattle infected with the mildly virulent RBT/1 virus strain. Two of the cows were infected when 23 and 36 weeks pregnant and both produced normal live calves 114 and 24 days after inoculation respectively. One of these calves, born 114 days after inoculation of the dam had a pre-colostrum serum antibody titre of 2.6 $\log_{10}SN_{50}$ to rinderpest virus. The other two cows infected at 23 and 27 weeks pregnant aborted 52 and 40 days after inoculation and there was no virological or serological evidence of foetal infection in these two cases. Except for the abortions, the clinical and virological features of the 4 cows were therefore similar to those previously noted for the RBT/1 strain (Plowright, 1963b). It is worth noting that parturition or abortion in the 4 cows occurred after the regression of viraemia and appearance of serum neutralizing antibodies in the dams. There is evidence that in utero transfer of antibodies to the foetus does not occur in cattle (Brambell, 1970). The presence of neutralizing antibodies in the pre-colostrum serum of the calf born 103 days after the mother's clinical recovery from rinderpest strongly suggests that the calf was infected in utero.

The 6 cows infected with the moderately virulent RBKK virus isolate reacted with a moderately severe disease characterized by fever, mouth lesions and diarrhoea. Deaths did not occur in these cows. The animals recovered from the disease and 4 cows, infected when 12 to 23 weeks pregnant, aborted 23 to 35 days following the dams' clinical recovery. Although the abortions occurred after 3 weeks following the regression of viraemia and appearance of neutralizing antibodies in the cows, rinderpest virus antigens were detected in the aborted foetal tissues thereby providing strong evidence of transplacental infection with this isolate. The 5th cow aborted 2 days following the remission of fever and there was no serological and virological evidence of in utero infection in the foetus. The 6th cow produced a normal live calf 182 days following the mother's inoculation. There was also no evidence of in utero infection in the calf.

Cattle infected with the enhanced virulent RBKO strain reacted with a severe clinical disease and heavy acute deaths. The only cow which recovered clinically and virologically from the disease aborted 12 days later and rinderpest virus was recovered from several aborted foetal tissues and maternal vaginal fluids.

These findings confirm earlier reports by Jacotot (1931) that infection of pregnant cattle with rinderpest virus may lead to abortion and excretion of virus in foetal tissues and maternal vaginal discharges. Not all clinically affected animals aborted. There is evidence from these studies that a proportion of the foetuses that are infected _in utero_ survive to term and possess high titres of pre-sucking neutralizing antibodies to rinderpest virus at birth. The mechanism which triggered foetal expulsion in infected cattle is currently not known but is probably due to either foetal death following invasion by the virus or secondary disturbances in maternal health and placental function (Ragsdale, Brody, Thompson and Worstell, 1948; MacFarlane, Pennycuik and Thrift, 1957).

A number of viruses such as those of BVD and Akabane may infect cattle with no ill effects on the foetus or may produce congenital abnormalities, abortion, still births, or the birth of weak, under-sized calves (Kurogi _et al_, 1976; Duffell and Harkness, 1985). The stage of pregnancy at which the infection occurs is an important determinant of the outcome although other factors including the strain of the virus and the immunological status of the host may also be significant (Barlow, 1972). The response of the bovine foetus to infection of the dam with rinderpest virus does not seem to be influenced by the stage of pregnancy if the infection occurs after the 12th week of pregnancy.

A comparison of the clinical and virological responses of pregnant cattle to infection with the four viruses indicates that the mildly and moderately virulent strains of rinderpest virus which cause mild to moderate disease and from which most animals recover may be the most important in the causation of abortion in cattle. Incidentally these are the strains most commonly associated with endemic rinderpest in many countries. The highly virulent strains are less important in this context as most of the animals die early in the course of the disease before foetal infection takes place. The attenuated vaccine strains apparently do not cross the bovine placenta.

The dynamics of growth and decline of rinderpest virus in cattle contracting infection have been studied in animals experimentally infected by parenteral routes (Scott, 1959c; Liess and Plowright, 1964). These authors have shown that rinderpest virus generally disappears from the body within 2 to 3 weeks of an infectious episode. Our observations, however, support the claim by Curasson (1932) that pregnant cows exposed to rinderpest virus may abort after 3 weeks following recovery from the disease and that foetal tissues and vaginal discharges from such animals contain virus. These findings indicate that the pattern and duration of virus growth and disappearance in pregnant cows may be prolonged and therefore differ from that in non-pregnant cattle. The prolonged presence of rinderpest virus in pregnant cattle may be of great significance with regard to the disease control and quarantine measures which, currently, are based on the fact that rinderpest virus does not persist in the body of an infected animal for more than 2 to 3 weeks.

TABLE 2.1 PATHOGENICITY FOR CATTLE OF DIFFERENT RINDERPEST VIRUSES

Virus	Fever	Mouth Lesions	Diarrhoea	Death	Status	Reference
Lapinized	-	-	-	-	Attenuated	Brotherston, 1951
KAG	+	-	-	-	Moderately attenuated	Daubney, 1947
RBT/1	+	+	$\pm$	-	Mildly virulent	Plowright, 1963b
RBKK	+	+	+	$\pm$	Moderately virulent	Wamwayi _et al_, 1989
RBKO	+	+	+	+	Highly virulent	Plowright and Ferris, 1959b

TABLE 2.2 CLINICAL RESPONSES IN CATTLE INFECTED WITH KAG VIRUS

Animal No.	Incubation period (days)	Duration of fever (days)	Day of delivery post inoculation
Z793	4	4	54
Z794	-	-	27
Z795	3	3	14
Z796	4	2	56

TABLE 2.3 ANTIBODY TITRES ($Log_{10}SN_{50}$) IN COWS INFECTED WITH KAG VIRUS

Animal No.	Days after inoculation 7	14
Z793	2.3	3.2
Z794	2.6	3.5
Z795	2.0	3.5
Z796	2.6	3.8

TABLE 2.4 LACK OF EVIDENCE OF FOETAL INFECTION IN CATTLE INFECTED WITH KAG VIRUS

Cow No.	Age* of gestation when infected	Maternal antibody+ at birth	Pre-colostrum calf antibody+	Calf antibody 2 days after birth
Z793	32	3.2	<0.3	2.6
Z794	35	3.5	<0.3	3.6
Z795	37	3.5	<0.3	2.9
Z796	32	3.2	<0.3	3.2

* = Age in weeks
\+ = $Log_{10}SN_{50}$

TABLE 2.5 CLINICAL RESPONSES IN CATTLE INFECTED WITH RBT/1 VIRUS

Cow No.	Incubation period (days)	Duration of fever (days)	Mouth Lesions	Diarrhoea	Abortion or normal birth
A13	3	4	+	-	C
A14	3	5	-	-	A
A18	3	5	+	-	A
A20	3	5	+	-	C

A = Aborted
C = Liveborn calf

TABLE 2.6 EVIDENCE OF FOETAL INFECTION IN CATTLE INFECTED WITH RBT/1 VIRUS

Cow No.	Day aborted or born post recovery	Maternal antibody* at birth or abortion	Pre-colostrum calf antibody*
A13	13C	2.9	<0.3
A14	41A	3.5	Nt
A18	29A	3.2	Nt
A20	103C	2.9	2.6

* = Antibody titre in $Log_{10}SN_{50}$
A = Aborted
C = Liveborn calf
Nt = Not tested

TABLE 2.7 VIRAEMIA* IN CATTLE INFECTED WITH RBT/1 VIRUS

	Days relative to onset of fever									
Cow No.	-1	0	1	2	3	4	5	6	7	8
A13	<0.4	<0.4	0.4	0.4	1.6	2.4	2.0	0.4	<0.4	Nt
A14	<0.4	Nt	0.4	1.0	1.2	2.2	1.2	0.4	0.4	<0.4
A18	<0.4	0.8	1.0	Nt	2.4	2.6	1.8	0.4	0.4	<0.4
A20	<0.4	0.4	0.8	0.4	2.0	2.6	1.8	<0.4	<0.4	<0.4
Median	<0.4	0.4	0.6	0.4	1.8	2.5	1.9	0.4	<0.4	<0.4

* = Virus titre in $Log_{10}TCED_{50}$ per ml of blood

Nt = Not tested

TABLE 2.8 ANTIBODY TITRES ($Log_{10}SN_{50}$) IN CATTLE INFECTED WITH RBT/1 VIRUS

	Days after inoculation	
Animal No.	7	14
A13	2.6	4.2
A14	3.9	4.0
A18	2.9	4.2
A20	2.3	3.9

TABLE 2.9 CLINICAL RESPONSES IN CATTLE INFECTED WITH RBKK VIRUS

Cow No.	Route of inoculation	Incubation period (days)	Duration of fever	Mouth Lesions	Diarrhoea
X3	IV	4	12	+	+
X19	IV	5	5	+	-
X93	C	10	6	+	+
X94	C	9	7	+	-
X106	SC	6	5	+	-
X110	SC	4	4	+	-

C = Contact infection

TABLE 2.10 VIRAEMIA IN CATTLE INFECTED WITH RBKK VIRUS

Days* of viraemia	Cow numbers X3	X19	X93	X94	X106	X110	Median
-2	<0.4+	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4
-1	0.4	<0.4	<0.4	<0.4	0.4	<0.4	<0.4
0	0.8	0.6	0.4	<0.4	0.4	0.6	0.5
1	0.8	1.0	0.8	0.4	0.8	1.0	0.8
2	1.4	0.8	ND	1.0	1.0	0.8	1.0
3	ND	1.4	1.2	1.4	ND	ND	1.4
4	ND	ND	1.6	1.4	ND	ND	1.5
5	0.8	ND	0.8	ND	1.0	ND	0.8
6	<0.4	0.4	<0.4	0.6	<0.4	ND	<0.4
7	<0.4	<0.4	<0.4	<0.4	<0.4	ND	<0.4
8	<0.4	<0.4	<0.4	<0.4	<0.4	ND	<0.4

+ = Virus titre in $Log_{10}TCED_{50}$ per ml of blood

* = Days relative to onset of pyrexia

ND = Not determined

TABLE 2.11 OCULAR SECRETION OF VIRUS BY CATTLE INFECTED WITH RBKK VIRUS

Days* of virus secretion	Cow numbers						
	X3	X19	X93	X94	X106	X110	Median
-1	0.4+	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4
0	0.8	1.0	<0.4	<0.4	0.8	0.8	0.8
1	1.6	1.6	1.0	<0.4	1.2	1.2	1.2
2	1.6	1.8	1.4	1.6	1.4	1.6	1.6
3	1.4	NT	1.6	1.8	1.4	NT	1.5
4	1.8	2.2	1.8	2.0	2.0	1.8	1.9
5	0.8	1.0	0.8	1.0	1.4	<0.4	0.9
6	<0.4	0.4	<0.4	<0.4	1.0	<0.4	<0.4
7	<0.4	0.4	<0.4	<0.4	<0.4	<0.4	<0.4
8	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4

+ = Virus titre in $Log_{10}TCED_{50}$ per swab

* = Days relative to onset of pyrexia

NT = Not tested

TABLE 2.12 VAGINAL SECRETION OF VIRUS BY CATTLE INFECTED WITH RBKK VIRUS

Days* of virus secretion	Cow numbers X3	X19	X93	X94	X106	X110	Median
-1	<0.4+	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4
0	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4
1	<0.4	0.4	<0.4	<0.4	<0.4	<0.4	<0.4
2	1.0	1.0	<0.4	<0.4	<0.4	0.8	0.6
3	1.4	1.6	1.2	1.1	1.4	1.6	1.4
4	1.6	1.8	1.6	1.2	1.4	1.4	1.5
5	1.4	<0.4	1.4	<0.4	1.6	1.0	1.2
6	1.0	<0.4	1.2	1.0	0.8	<0.4	0.9
7	<0.4	<0.4	0.4	<0.4	<0.4	<0.4	<0.4
8	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4

+ = Virus titre in $Log_{10}TCED_{50}$ per swab

* = Days relative to onset of pyrexia

TABLE 2.13 EVIDENCE OF FOETAL INFECTION IN COWS INFECTED WITH RBKK VIRUS

Cow No.	Days* onset of viraemia	Day[1] aborted	Maternal[2] antibody	Foetal[3] antibody	Antigen[4] presence
X3	-1	30	3.0	-	++
X19	0	35	3.0	-	++
X93	0	23	3.0	-	++
X94	1	2	2.4	-	-
X106	-1	LC	3.2	-	NT
X110	0	30	3.4	-	+++

* = Days relative to onset of pyrexia

1 = Day aborted post recovery

2 = At time of abortion or birth in $Log_{10}SN_{50}$

3 = Foetal and pre-colostral antibody in $Log_{10}SN_{50}$

4 = In the foetus

LC = Liveborn calf

NT = Not tested

TABLE 2.14 ANTIBODY TITRES ($LOG_{10}SN_{50}$) IN CATTLE INFECTED WITH RBKK VIRUS

	Days after inoculation		
Cow No.	7	14	21
X3	2.8	4.2	4.2
X19	2.1	3.9	4.0
X93	2.8*	3.9	Nt
X94	2.5*	3.6	Nt
X106	2.5	4.2	4.0
X110	2.4	3.6	3.9

* = Antibody titres after onset of fever

TABLE 2.15 CLINICAL RESPONSES IN CATTLE INFECTED WITH RBKO VIRUS

Cow No.	Incubation period (days)	Duration of fever (days)	Mouth lesions	Diarrhoea	Abortion	Maternal deaths
Z413	3	3	++	-	-	+ (3)
Z414	3	5	++	++	+	-
Z418	3	2	-	-	-	+ (3)
Z420	3	2	++++	-	-	+ (3)

( ) = Day animal died following onset of fever

TABLE 2.16 RELATIONSHIPS BETWEEN PYREXIA, VIRAEMIA, VIRUS IN VAGINAL SECRETIONS AND ABORTION IN COW Z414 INFECTED WITH RBKO VIRUS STRAIN

Days relative to onset of fever	Fever (^oC)	Viraemia*	Vaginal secretion of virus	Abortion
-2	38.3	<0.4	<0.4	
-1	38.8	0.8	<0.4	
0	40.1	1.8	<0.4	
1	40.2	2.0	0.8	
2	40.5	2.6	2.0	
3	40.2	2.5	1.8	
4	40.4	0.8	0.8	
5	38.3	0.4	<0.4	
6	38.5	<0.4	<0.4	
7	37.9	<0.4	<0.4	
8	38.1	<0.4	<0.4	
19	38.1	<0.4	2.4	+
20	38.2	<0.4	1.2	
21	38.0	<0.4	<0.4	

* = Virus titre in $Log_{10}TCED_{50}$ per ml

TABLE 2.17 VIRUS TITRES IN TISSUES OF A FOETUS ABORTED BY RBKO VIRUS-INFECTED COW

Field tissue	Virus titre*
Cotyledon	2.6
Spleen	2.0
Lung	1.8
Abomasum	1.8
Meconium	1.8
Liver	1.4
Chorioallantoic membrane	1.6
Kidney	1.4
Testis	<1.4

*$Log_{10}TCED_{50}$ per g

TABLE 2.18 ANTIBODY TITRES ($Log_{10}SN_{50}$) IN A COW INFECTED WITH RBKO VIRUS

	Days after inoculation			
Cow No.	7	14	21	28
Z414	2.8	3.9	4.2	4.2

TABLE 2.19 **RELATIONSHIP BETWEEN GESTATIONAL AGE AND FOETAL RESPONSE TO INFECTION OF PREGNANT CATTLE WITH RINDERPEST VIRUS**

a. NORMAL BIRTHS

With evidence of foetal infection			No evidence of foetal infection		
Cow No.	Gestation age* at infection	Day+ born	Cow No.	Gestation age* at infection	Day+ born
A20	23	114	Z793	32	54
			Z794	35	27
			Z795	37	14
			Z796	32	56
			A13	36	24
			X106	12	182

* = Age in weeks
\+ = Days after infections

b. ABORTIONS

With evidence of foetal infection			No evidence of foetal infection		
Cow No.	Gestation age* at infection	Day+ aborted	Cow No.	Gestation age* at infection	Day+ aborted
X3	12	47	A14	23	52
X19	23	48	A18	27	40
X93	17	41	X94	23	17
X110	23	42			
Z414	34	23			

* = Age in weeks
\+ = Days after infection

TABLE 2.20 COMPARATIVE RESPONSES OF PREGNANT COWS INFECTED WITH DIFFERENT STRAINS OF RINDERPEST VIRUS

	Virus strains			
Parameter	KAG	RBT/1	RBKK	RBKO
Days of fever	2-4	4-5	4-12	2-5
Median viraemia	ND	6.0	6.5	7.0
Mouth lesions	0/4	3/4	5/6	3/4
Diarrhoea	0/4	0/4	4/6	1/4
Mortality	0/4	0/4	0/6	3/4
Abortions	0/4	2/4	5/6	1/1
Virus presence	0/4	0/4	0/6	1/1
Antigen presence	0/4	0/4	4/6	ND
Antibody presence	0/4	1/4	0/6	ND

ND = Not determined

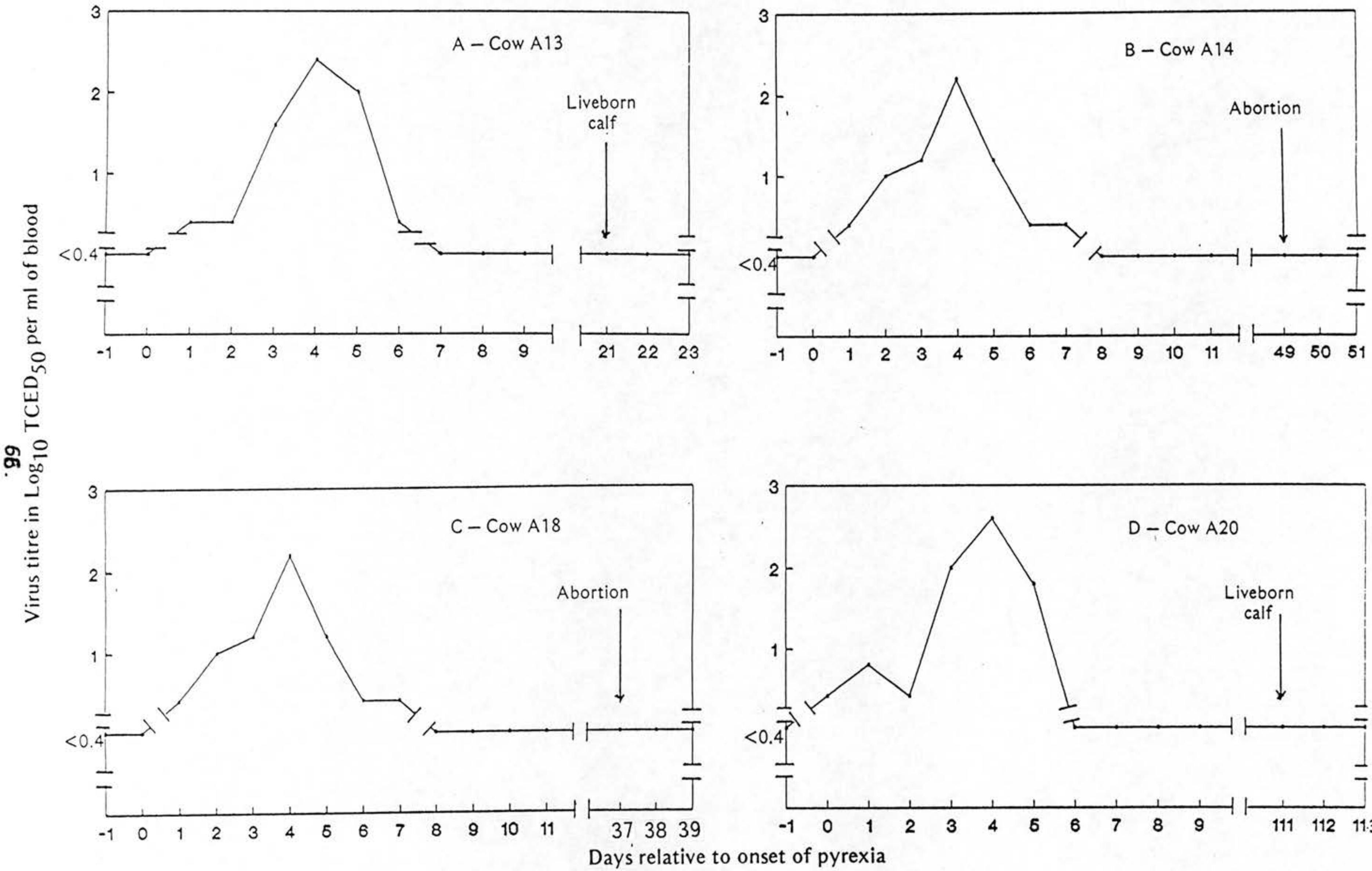

FIGURE 2.1 CORRELATION BETWEEN VIRAEMIA AND ABORTION IN CATTLE INFECTED WITH RBT/1 VIRUS

FIGURE 2.2 RETAINED PLACENTA IN A COW INFECTED WITH RBKK VIRUS.

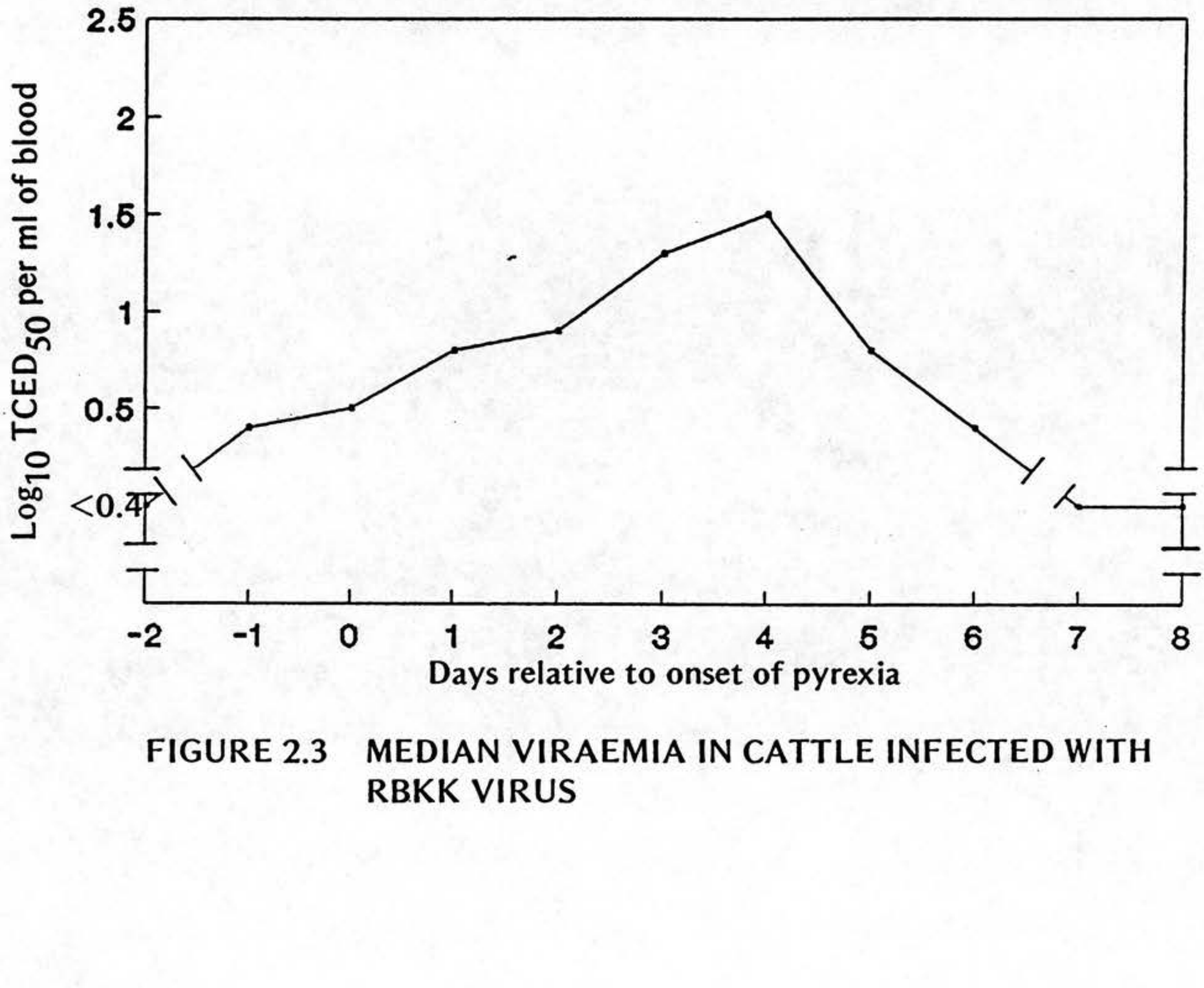

FIGURE 2.3 MEDIAN VIRAEMIA IN CATTLE INFECTED WITH RBKK VIRUS

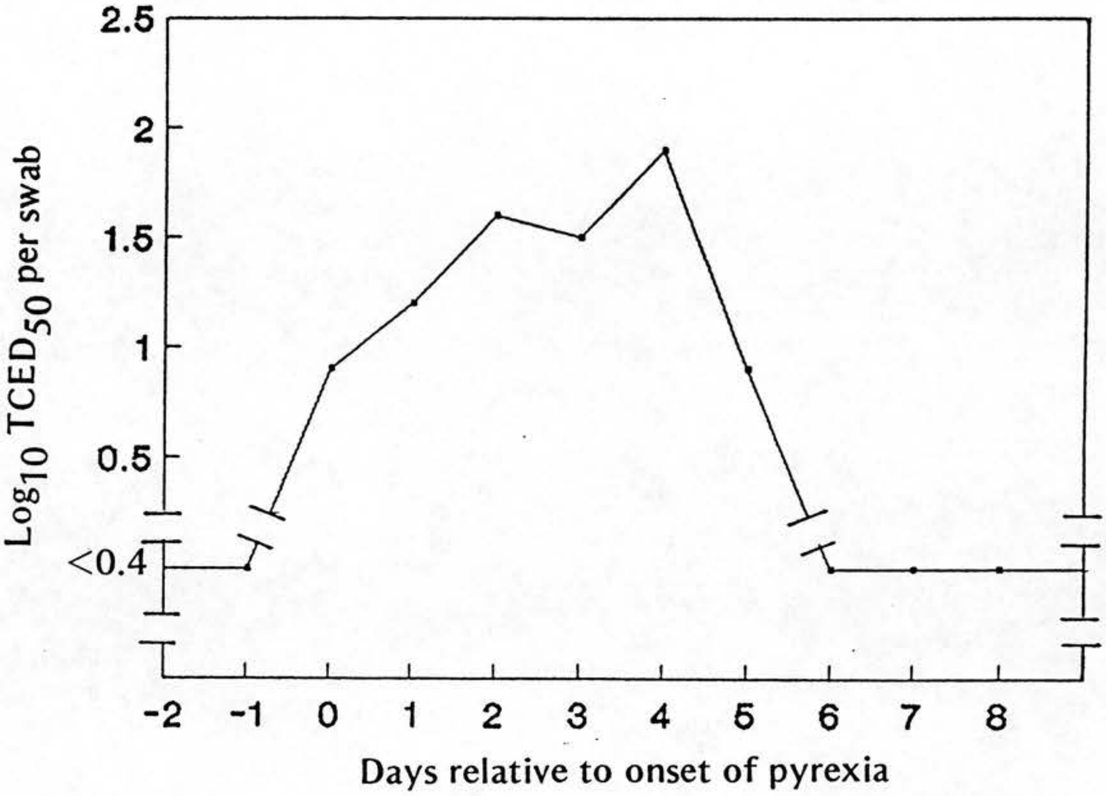

FIGURE 2.4 OCULAR SECRETION OF VIRUS BY CATTLE INFECTED WITH RBKK VIRUS

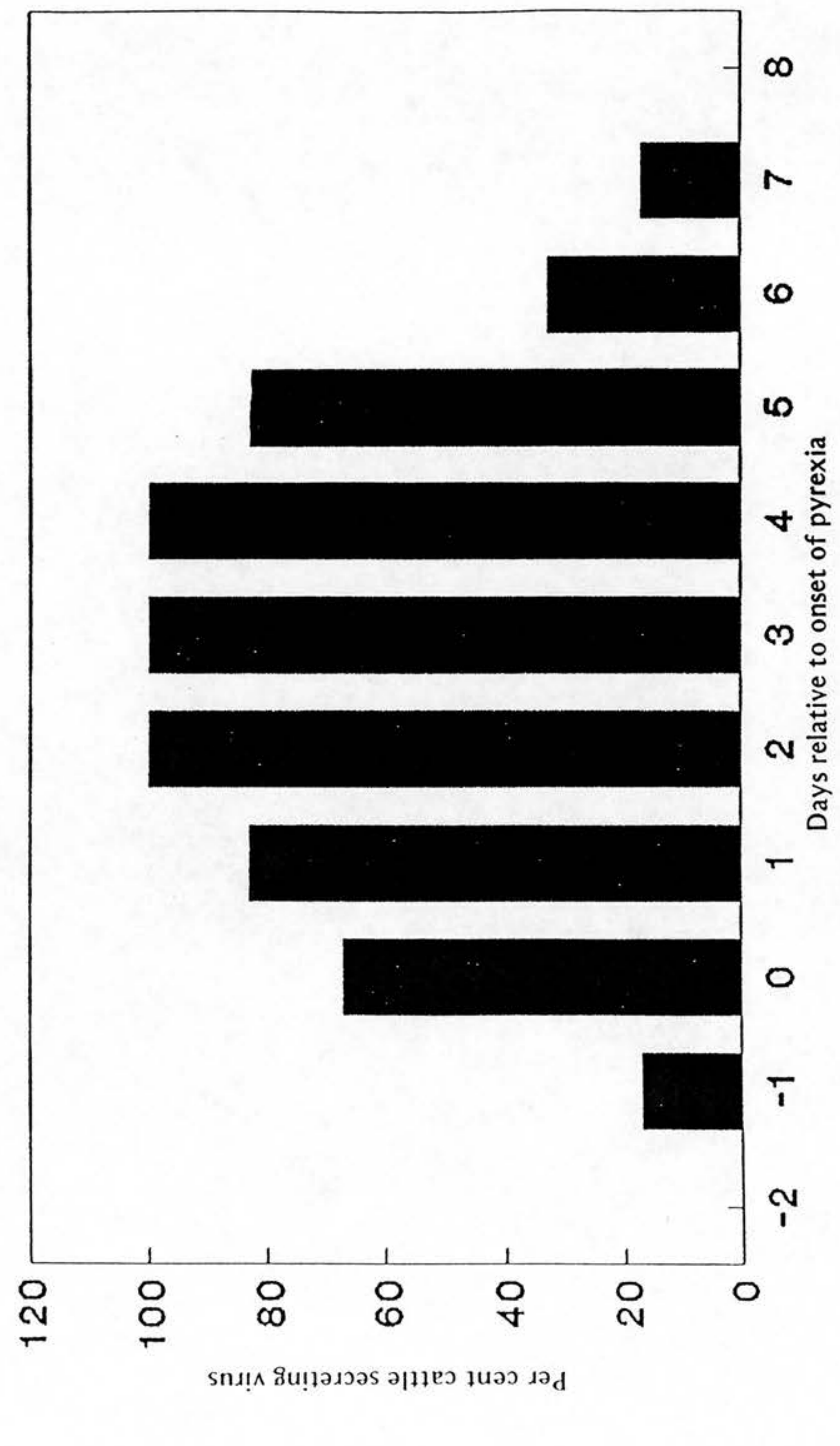

FIGURE 2.5 OCULAR SECRETION OF VIRUS BY CATTLE INFECTED WITH RBKK VIRUS

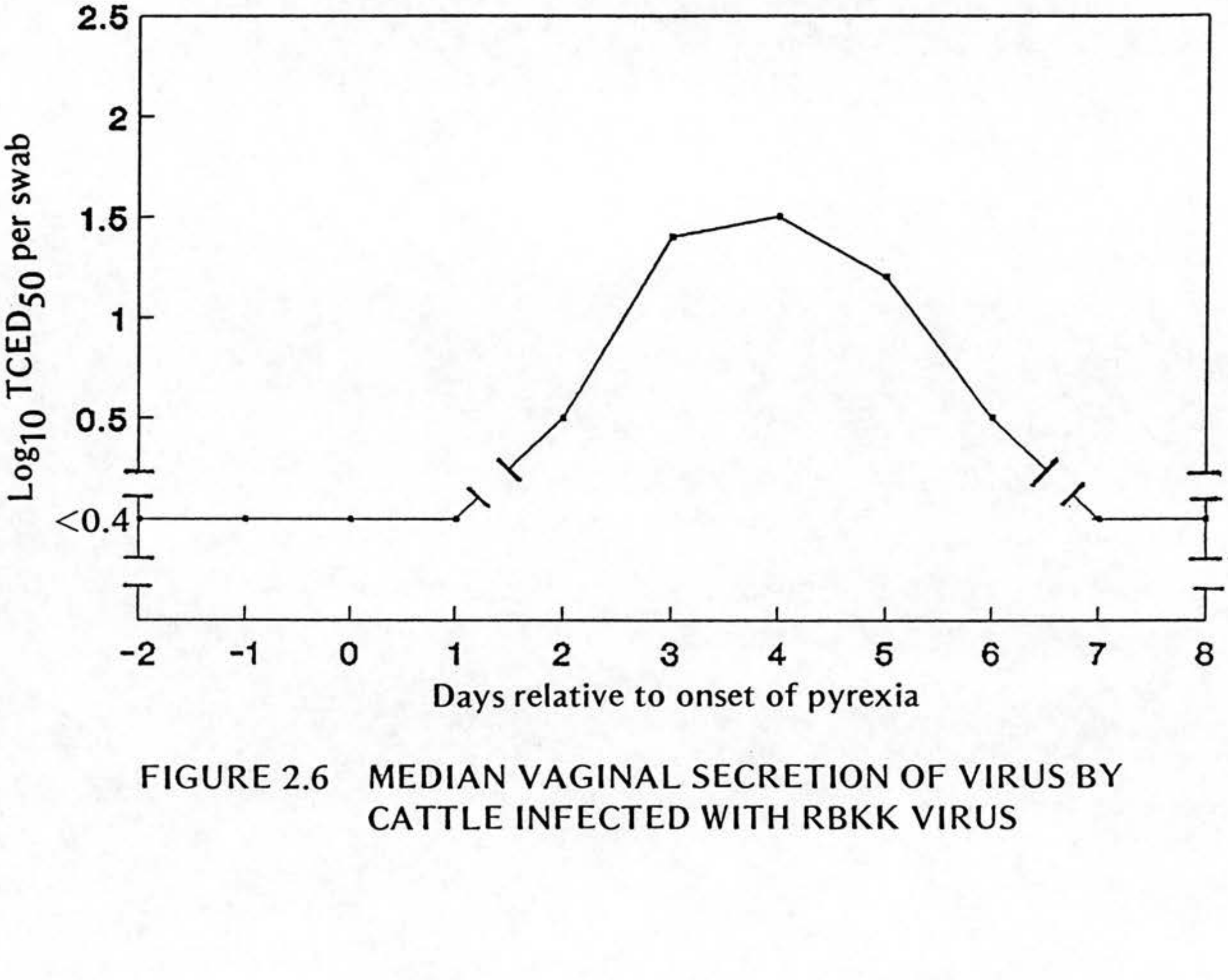

FIGURE 2.6 MEDIAN VAGINAL SECRETION OF VIRUS BY CATTLE INFECTED WITH RBKK VIRUS

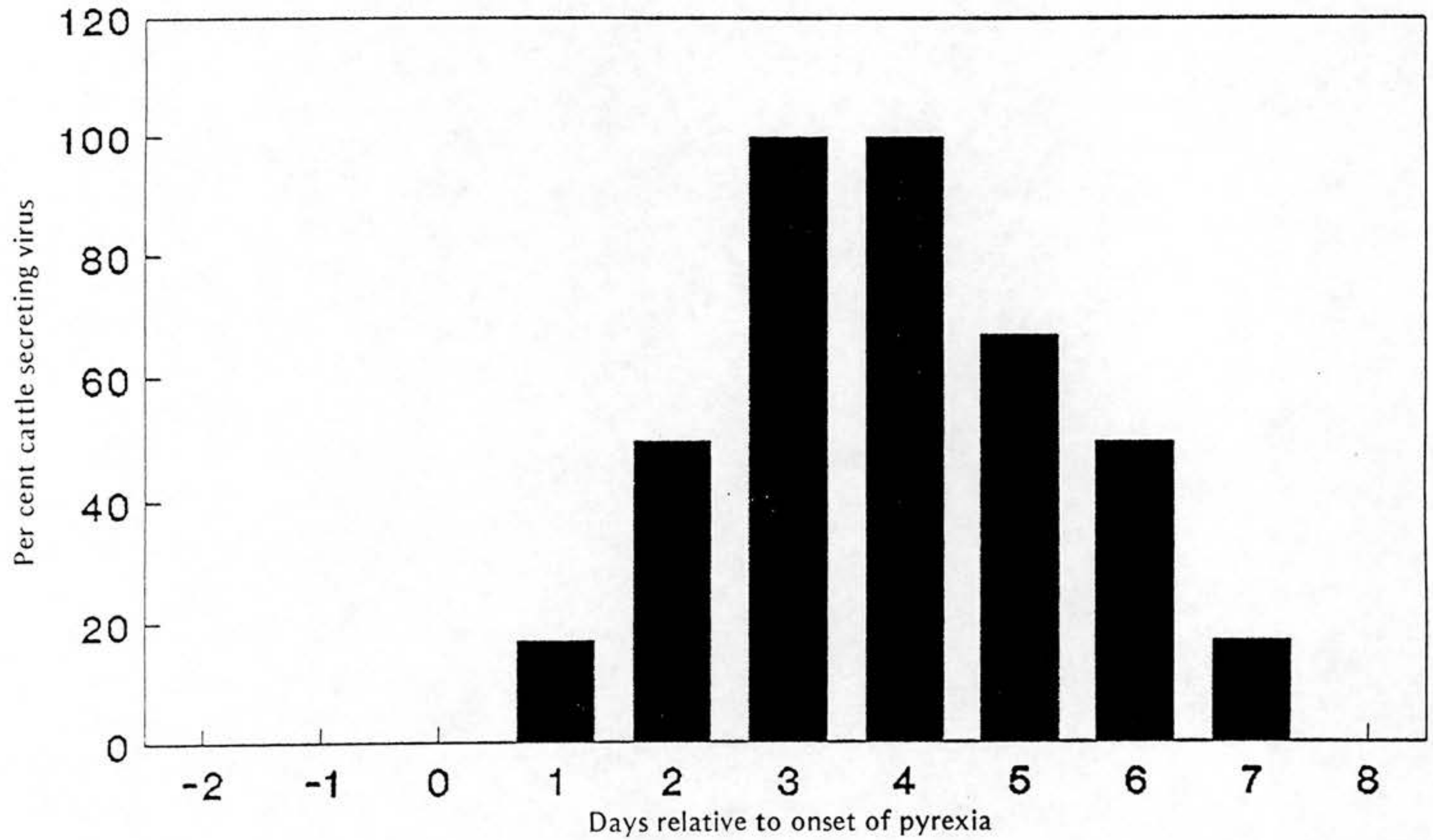

FIGURE 2.7 VAGINAL SECRETION OF VIRUS BY CATTLE INFECTED WITH RBKK VIRUS

FIGURE 2.8 SUBCUTANEOUS OEDEMA AND CONGESTION IN AN ABORTED FOETUS FROM A COW INFECTED WITH RBKK VIRUS.

FIGURE 2.9 ORAL HYPERAEMIA AND EROSIONS IN A FOETUS ABORTED BY A COW INFECTED WITH RBKK VIRUS.

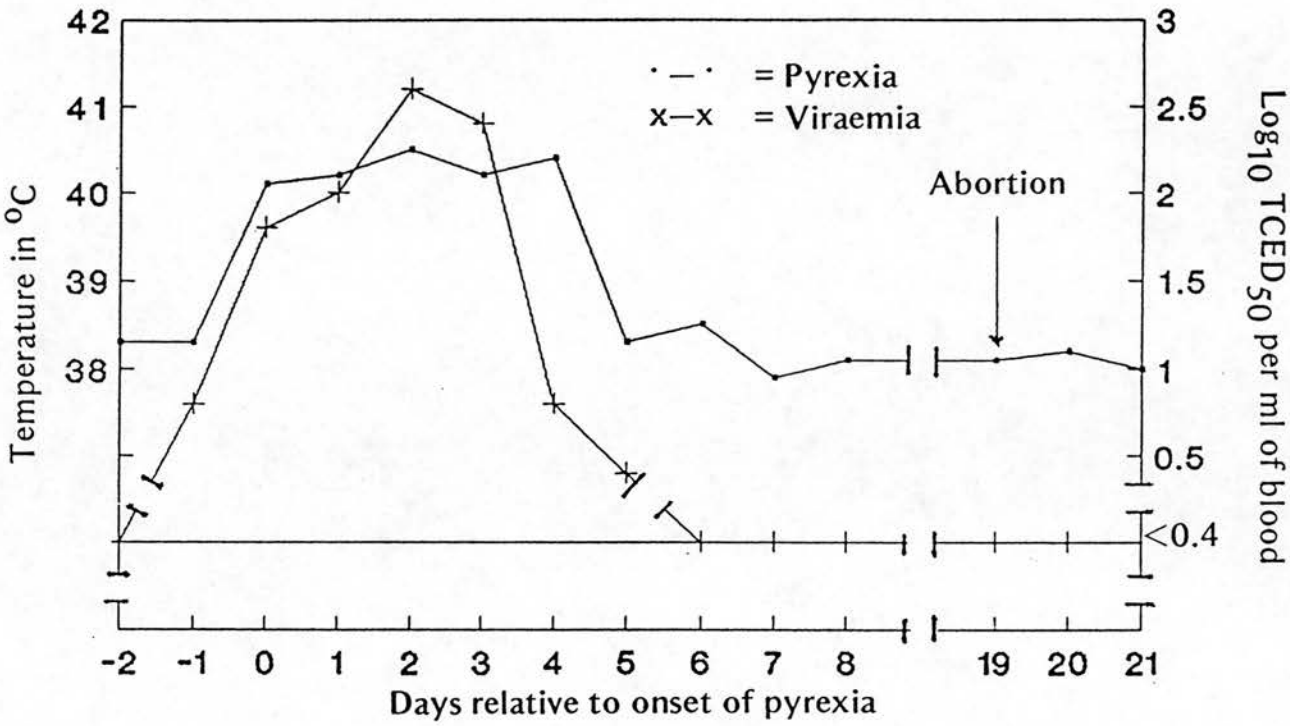

FIGURE 2.10 CORRELATION BETWEEN PYREXIA, VIRAEMIA AND ABORTION IN COW Z414 INFECTED WITH RBKO VIRUS

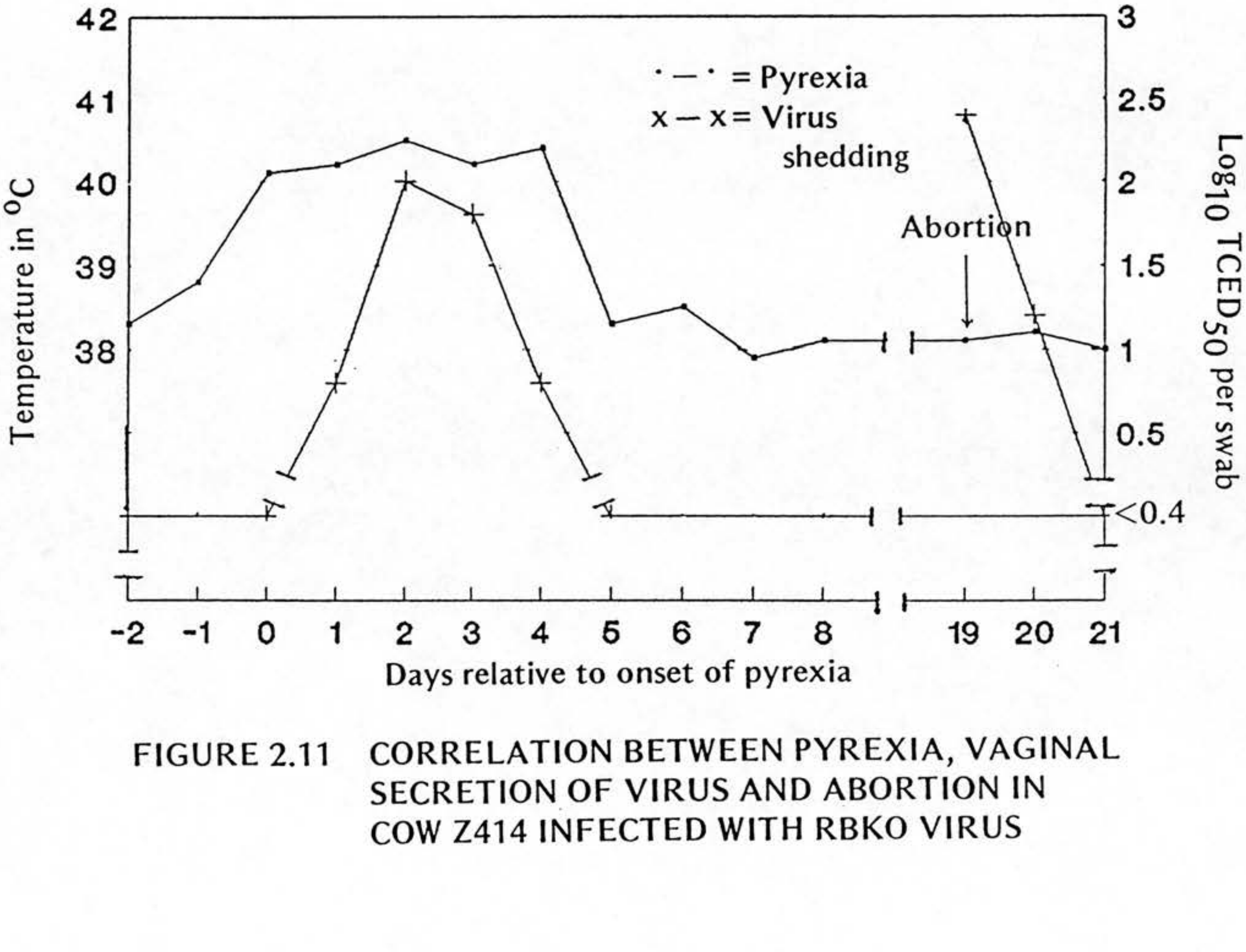

FIGURE 2.11 CORRELATION BETWEEN PYREXIA, VAGINAL SECRETION OF VIRUS AND ABORTION IN COW Z414 INFECTED WITH RBKO VIRUS

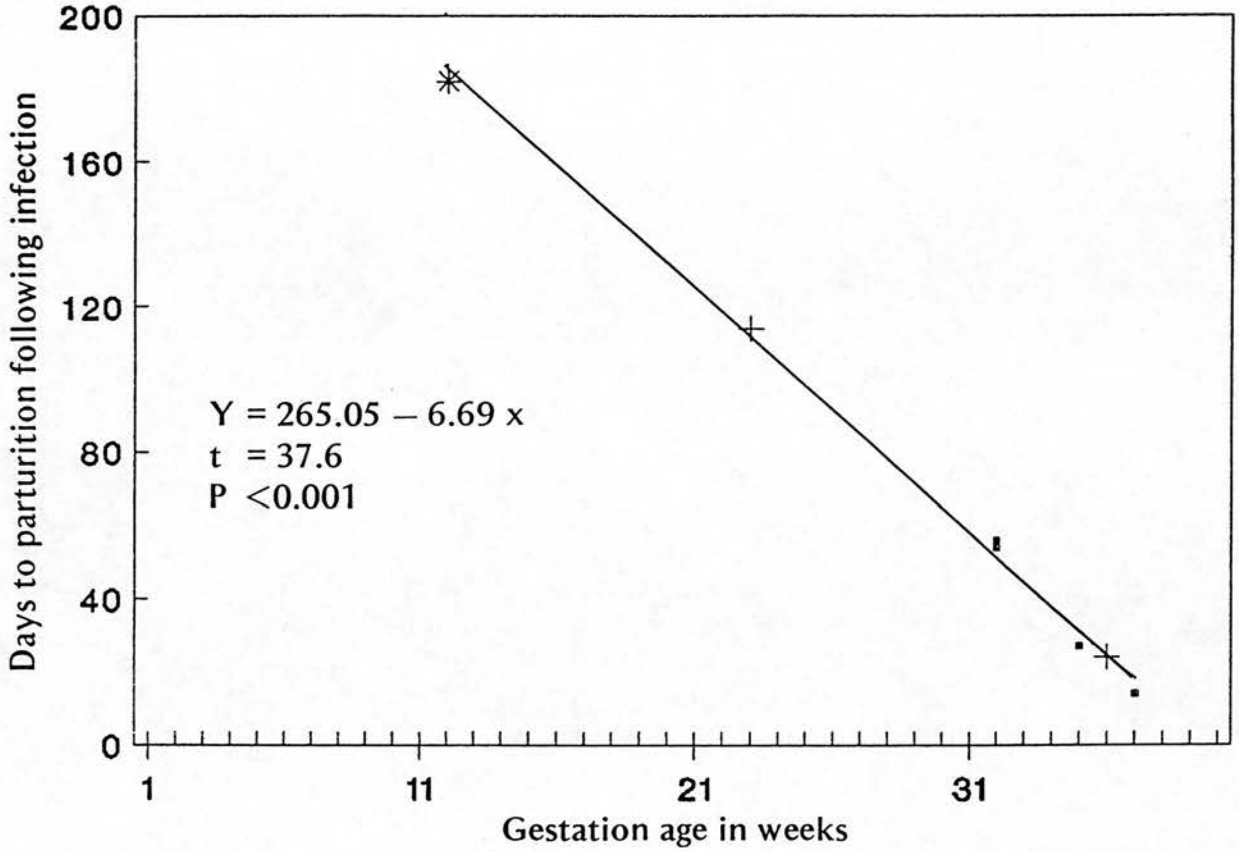

FIGURE 2.12 RELATIONSHIP BETWEEN GESTATION AGE AT INFECTION AND NORMAL PARTURITION IN CATTLE INFECTED WITH RINDERPEST VIRUS

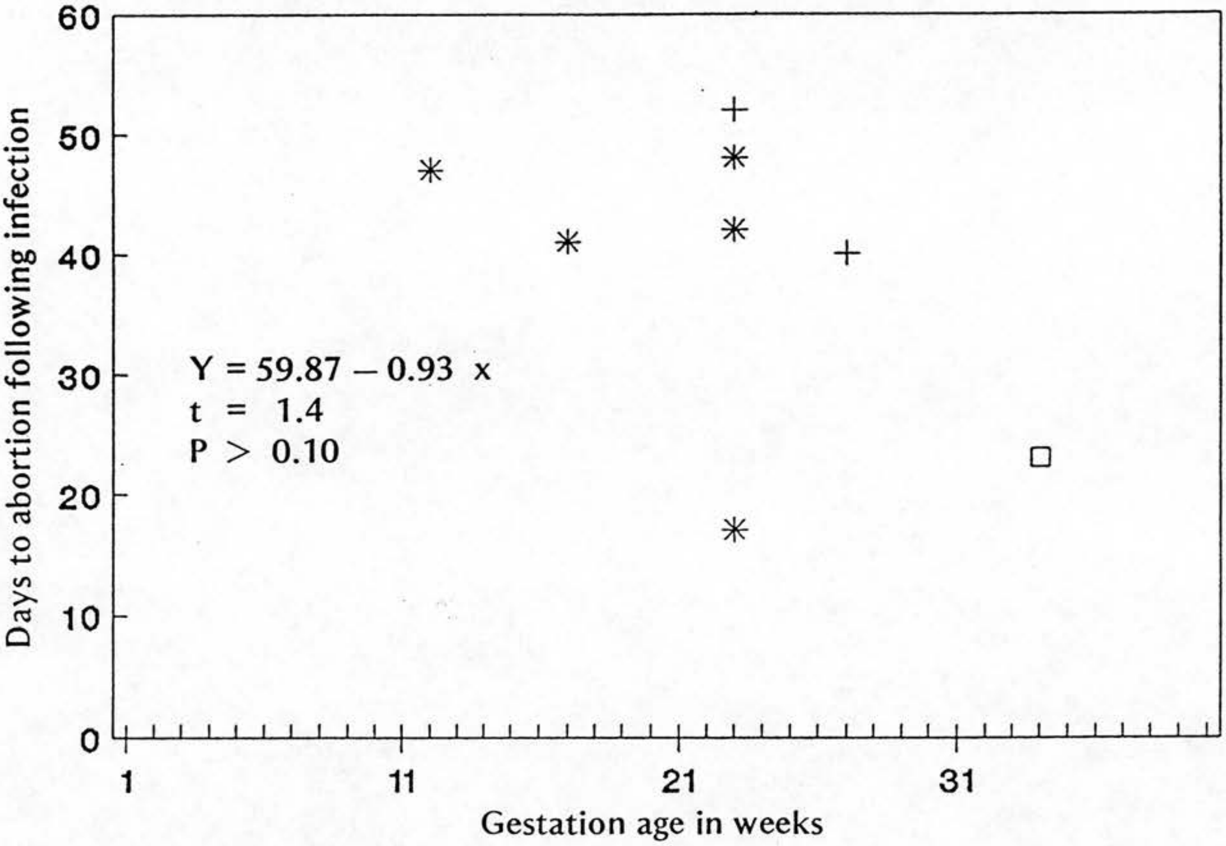

FIGURE 2.13 RELATIONSHIP BETWEEN GESTATION AGE, TIME OF INFECTION AND ABORTION IN CATTLE INFECTED WITH RINDERPEST VIRUS

CHAPTER THREE

ONSET OF FOETAL INFECTION IN RINDERPEST INFECTED COWS

INTRODUCTION

A wide variety of viruses infect adult cattle and, not surprisingly, some transmit to and infect the foetus (Kendrick and Straub, 1967; Sattar, Bohl and Trapp, 1967; Classick and Fernelius, 1970; Swift and Kennedy, 1972; Braun, Osburn and Kendrick, 1973; Hubbert, Bryner, Fernelius, Frank and Estes, 1973). In their review of the viral infections of the bovine foetus Hubbert and others (1973) reported that 14 different viruses including rinderpest virus were known to infect the bovine foetus or placenta or were associated clinically with foetal death, abortion or other syndromes of the foetus. Data on the time of transplacental transmission of viral agents from the dam to the foetus are conspicuous by their absence. It is not known whether such transmission occurs at the same time as virus multiplication in the organs of the dam. There are indications from the data in Chapter Two that foetuses from animals that die or abort during or soon after the acute phase of rinderpest infection are devoid of virus while several of those that are aborted 10 or more days following the remission of viraemia show evidence of transplacental infection.

The following sequential experiments were therefore conducted in an attempt to follow the virological events in the foetus in the first 21 days of rinderpest virus infection of cattle after the third month of gestation.

MATERIALS AND METHODS

Infection of cattle with RBKK virus

Two groups of 6 adult Zebu cows, 17 to 35 weeks pregnant were used. Each cow in the first group was inoculated subcutaneously with 2 ml of a 20 per cent spleen suspension harvested on the 3rd day of pyrexia from a cow previously infected with blood from an RBKK infected cow. The infectivity titre in the suspension was $10^{1.6}TCED_{50}$ per g. Each animal in the second group was inoculated subcutaneously with tissue culture fluids of the 2nd BK-passage RBKK strain of rinderpest virus, the infectivity titre in the inoculum being $10^{2.6}TCED_{50}$ per ml.

Infection of cattle with RBKO virus

Eight Zebu cows aged 3 to 6 years and 20 to 34 weeks pregnant were inoculated subcutaneously with 2ml spleen suspension containing $10^{5.2}$ cattle ED_{50} of the highly virulent RBKO strain of rinderpest virus.

Post-infection procedures

The three groups of cattle were housed separately in disease-secure accommodation and provided hay and water *ad libitum*.

The animals were examined daily for clinical signs; and blood in EDTA and vaginal swabs were collected daily. One cow from each of the two groups infected with the RBKK virus was killed at intervals of 3 days from the 2nd day after the onset of fever and both maternal and foetal blood in EDTA, foetal thymus, lung, lymph nodes, kidney, liver, cotyledons, foetal membranes and allantoic, amniotic, pleural and peritoneal fluids were collected. Similar tissues were collected post-mortem from the cows that died or were killed following infection with the RBKO virus.The samples were processed and examined for the presence of rinderpest virus, precipitating antigens and neutralizing antibodies as previously described (Chapter Two).

RESULTS

RBKK virus infected cows

Clinical signs

The incubation period in cattle inoculated with infected spleen suspension ranged from 4 to 6 days (Table 3.1). The duration of fever in the animals that survived beyond the 5th day after the onset of fever was 5 to 10 days. Two cows (Z90 and Z96) had bi-phasic temperature reactions with the first phase lasting 6 days in both cases. Giemsa-stained blood and lymph node smears from the latter two cows were examined and found negative for babesia, anaplasma and theileriosis.

Four out of the 6 cows inoculated with the cell-culture passaged virus developed fevers of greater than 39.5^{o}C on the 5th day of inoculation while the other two cows became pyrexic on the 6th day (Table 3.1). The duration of fever when considered without the animal that was killed on the 2nd day after the onset of fever was 5 to 7 days.

The pattern of clinical signs in all the 12 animals was similar to that already described in detail for cattle infected with the RBKK virus (Chapter Two). Serous to seromucoid oculo-nasal discharges occurred in all the animals. Mouth lesions were observed in 4 out of the 6 cows inoculated with the spleen suspension but were present in 5 of the 6 animals exposed to the cell culture-passaged virus. Diarrhoea developed in 1 cow that received the infected spleen and was observed in 2 animals that received culture passaged virus.

Virus in the dams

Viraemia was first detected in 4 out of the 6 cattle inoculated with infective RBKK spleen suspension on the day of onset of fever but all cows were viraemic by the 1st day after the onset of fever (Table 3.2). Three cows inoculated with culture-passaged virus were viraemic on the day preceding the onset of fever while all cattle infected with the culture-propagated virus had virus in the blood on the day of onset of fever. The duration of viraemia in cattle that received spleen suspension and survived beyond the 5th day of fever was 5 to 8 days with a median viraemic period of 6.3 days. In cattle infected with the culture-passaged virus the duration of viraemia was 7 to 9 days with a median of 8.2 days. Unfortunately and unexpectedly, there occurred at this time massive bacterial and fungal contaminations of cell cultures and attempts to isolate virus from foetal samples were unsuccessful. Rinderpest virus was however recovered in BK cells from the placentomes collected on day 11 following the onset of fever in cow Z90 infected with spleen suspension (Table 3.2). Rinderpest virus was not isolated from other foetal blood, tissues and fluids.

Antigen detection

Rinderpest virus antigens were demonstrated in foetal thymus, spleen and mesenteric lymph nodes collected from a cow inoculated with the culture-passaged virus and killed on day 17 after the onset of fever (Table 3.2). Virus antigens were not detected in foetal tissues and fluids from cows killed before the 17th day after the onset of fever.

Serology

The development of neutralizing antibodies to rinderpest virus in adult cattle followed the typical pattern (Table 3.3) and was similar to that already recorded in cattle infected with the RBKK virus isolate. Antibodies were not detected in foetal blood, tissue exudates and amniotic, allantoic, pleural and peritoneal fluids. The levels of antibody development between the two groups of cattle infected with the two RBKK virus preparations were similar.

RBKO virus infected cows

Clinical signs

Five animals developed a fever of greater than 39.5^{o}C on the 4th day after inoculation while the remaining cows had fever on the 5th day after inoculation (Table 3.4). Pyrexia was in all cases accompanied by hyperaemia and congestion of the visible mucous membranes and a serous nasal and ocular discharge. The animals progressively developed severe clinical signs characteristic of rinderpest. Animals number Z36, Z54 and Z32 were respectively killed on the day of onset of fever and on days 2 and 5 following the onset of fever (Table 3.5). Animal No. 49 died on the 2nd day, following the onset of fever while animals number Z37 and Z44 died on the 3rd day. Animals number Z56 and Z51 died on the 4th and 5th days respectively.

Virus in the dams

Virus was first detected in the blood of 6 cows on the day preceeding the onset of fever and was also present in the remaining 2 cows on the day of fever (Table 3.4 and Figure 3.1). Viraemia was detected upto the 5th day after the onset of fever in animals that survived to that day (Figure 3.1).

Rinderpest virus was demonstrated in vaginal secretions from 5 cows on the day of onset of fever and from the remaining 3 animals on the 2nd day of pyrexia. The median peak titre of virus in the vaginal secretions was attained on the 2nd day of fever and declined steadily thereafter to trace levels by the 5th day following the onset of fever.

Virus in the foetuses

A low titre of rinderpest virus was first detected in the placentomes of one of the two foetuses obtained on the 3rd day after the onset of fever i.e. on the 5th day of viraemia in the dam (Table 3.5). Virus was thereafter demonstrated in the placentomes of foetuses obtained on the 4th and 5th days after the onset of fever. The highest virus titre of $10^{2.4}TCED_{50}$ per g was demonstrated in the placentomes from cow Z32 killed on the 5th day after the onset of fever.

Low titres of virus were demonstrated in blood and spleen of one of the 2 foetuses collected post-mortem on the 5th day following the onset of pyrexia. Virus was not demonstrated in the other foetal tissues and fluids examined.

Serology

Neutralizing antibodies to rinderpest virus were not detected in cattle sera collected on the 3rd day after inoculation but low levels were detected in 6 cows on the 6th day and in 2 cows on the 9th day after inoculation (Table 3.6).

Gross pathology

Ulcerative and haemorrhagic gastroenteritis was observed in the cows that died or were killed between the 3rd and 5th days following the onset of fever. There was congestion of the placentomes in the cows that were examined on days 2 and 3 while those examined on days 4 and 5 had both congestion and brownish colouration of the placentomes.

DISCUSSION

Cattle inoculated with infective spleen and culture-passaged RBKK virus did not show marked differences in the pattern and severity of their clinical signs. Both groups reacted with a disease characterized by fever, mild oral lesions, minimal diarrhoea and no mortalities.

On the other hand, the 8 cows infected with the RBKO virus strain suffered a severe disease that was manifested by extensive oral lesions, profuse diarrhoea and a high mortality rate. Rinderpest virus was isolated from the placentomes of 4 out of the 5 cows that were still alive on and after the 3rd day following the onset of fever and the amount of virus isolated from this organ increased steadily up to the 5th day following the onset of fever. It should be noted that the placentome here denotes the combined foetal cotyledon and maternal caruncle because attempts to separate the two structures were in many cases unsuccessful. It is therefore possible that the virus detected in this organ may have originated in the maternal part of the placentome.

Examination of cattle infected with the RBKO virus strain were not extended beyond the 9th day of inoculation as all cattle had either been killed or died by this day. The heavy early mortalities thus encountered rendered it difficult to assess the progressive development, distribution and duration of the virus in various foetal tissues. Chapter Two of this thesis records the isolation of rinderpest virus from the cotyledons of a foetus that was expelled 23 days following inoculation of the mother with the RBKO virus. These findings suggest that the RBKO virus strain may persist in foetal cotyledons for at least 10 days after the disappearance of virus in the maternal blood.

Only low levels of virus were detected in foetal blood and spleen from one of the two cows examined on the 5th day after the onset of fever. Virus was however detected in the placentomes of infected animals from the 3rd day following the onset of fever. This would indicate that transmission of virus from the dam to the foetus probably took place via the placenta since foetal infection was preceded by placental infection. The isolation of the virus from tissues of a foetus aborted 23 days after maternal infection with the RBKO virus strain (Chapter Two) suggests a gradual replication and distribution of virus in infected foetal tissues from the 7th day of maternal viraemia.

Rinderpest virus was recovered from the placentome of only one animal inoculated with the RBKK isolate on the 11th day following the onset of maternal fever. Attempts to isolate virus from foetus of the dams killed after the 11th day following the onset of fever were unsuccessful and this may have largely been due to the massive bacterial and fungal contamination of cell cultures at this stage.

The demonstration of rinderpest virus precipitating antigen in the foetal thymus, spleen and mesenteric lymph nodes of a foetus recovered on the 17th day following the onset of fever in cow Z68 indicates that foetal infection with the RBKK occurred by the 20th day of inoculation. The transmission of the virus from the mother to the foetus likewise occurred through the placenta because placental infection preceded foetal infection. Based on the speculative pathogenesis of RBKK virus infection in cow Z68 (Figure 3.2) the explanation for the failure to detect antibody to rinderpest virus in foetal blood and tissue exudates might be that the foetus was collected only a few days after its infection.

These findings, together with the observations in Chapter Two that tissues from foetuses expelled 2 to 7 weeks following clinical recovery of the dams from rinderpest infection contain virus and virus antigens suggest that rinderpest virus passes from maternal circulation to the placenta during the late viraemic stage in the cow and multiplies briefly in the placenta from where it passes into the foetus causing an infection which may kill the foetus with subsequent abortion. It is noteworthy that rinderpest virus was able to persist in the placentomes and other foetal tissues for a long time despite the presence of neutralising antibodies in the dam. This suggests that the placentome was not damaged with the passage of the virus through it. However, further studies to provide data correlating the growth of rinderpest virus and the immunological response of the bovine foetus to the virus should be undertaken.

TABLE 3.1 CLINICAL RESPONSES IN CATTLE INFECTED WITH RBKK VIRUS

Cow No.	Inoculum	Incubation period (days)	Days of fever	Mouth lesions	Diarrhoea
Z6	20% spl.	5	2	-	-
Z10	"	4	5	-	-
Z54	"	6	6	+	-
Z90	"	4	10	+	+
Z91	"	4	5	+	-
Z96	"	4	9	+	-
Z17	BK2	4	2	-	-
Z33	"	5	5	+	-
Z50	"	4	6	+	-
Z59	"	4	7	+	+
Z65	"	5	5	+	-
Z68	"	4	6	+	+

Spl = Infective spleen
BK2 = 2nd BK culture-passaged virus

TABLE 3.2 ONSET OF FOETAL INFECTION WITH RBKK VIRUS

Cow No.	Onset* of viraemia	Days of viraemia	Day* killed	Foetal infection Placenta	Spleen	Lymph node	Thymus
Z6	0	3	2	-	-	-	-
Z10	0	6	5	-	-	-	-
Z54	1	5	8	-	-	-	-
Z90	1	8	11	v	-	-	-
Z91	0	7	14	-	-	-	-
Z96	0	7	17	-	-	-	-
Z17	-1	4	2	-	-	-	-
Z33	0	6	5	-	-	-	-
Z50	-1	8	8	-	-	-	-
Z59	0	7	11	-	-	-	-
Z65	0	8	14	-	-	-	-
Z68	-1	9	17	ag	ag	ag	ag

* = Relative to onset of fever
ag = Antigen detected
v = Virus detected

TABLE 3.3 ANTIBODY TITRES ($Log_{10}SN_{50}$) IN CATTLE INFECTED WITH RBKK VIRUS

Animal No.	Days after inoculation 7	14	21
Z6	1.0	NT	NT
Z10	1.2	NT	NT
Z54	0.8	3.0	NT
Z90	<0.3	2.8	NT
Z91	0.8	2.8	NT
Z96	0.8	3.0	2.8
Z17	NT	NT	NT
Z33	1.0	NT	NT
Z50	1.0	NT	NT
Z59	<0.3	3.0	NT
Z65	1.0	2.8	NT
Z68	1.2	3.0	4.0

NT = Not tested

TABLE 3.4 CLINICAL RESPONSES OF CATTLE INFECTED WITH RBKO VIRUS

Cow No.	Incubation period (days)	Days of fever	Onset* of viraemia	Duration of viraemia	Mouth lesions	Diarrhoea
Z32	4	4	-1	7	+	+
Z36	3	1	-1	2	-	-
Z37	3	4	0	NT	+	-
Z44	3	3	-1	NT	+	-
Z49	3	3	0	3	-	-
Z51	4	3	-1	NT	+	+
Z54	3	3	-1	NT	+	+
Z56	4	4	-1	7	+	-

* = Relative to onset of fever
NT = Not tested

TABLE 3.5 ONSET OF FOETAL INFECTION WITH RBKO VIRUS

Day after onset of fever	**Animal Number** Killed	Died	Placentome	**Virus titres*** Spleen	Blood
0	Z36		<1.4	<1.4	<0.4
2	Z54		<1.4	<1.4	<0.4
		Z49	<1.4	<1.4	<0.4
3		Z37	<1.4	<1.4	<0.4
		Z44	1.4	<1.4	<0.4
4		Z56	1.6	<1.4	<0.4
5	Z32		2.4	1.4	0.4
		Z51	2.0	<1.4	<0.4

* = $Log_{10}TCED_{50}$ per ml or g

TABLE 3.6 ANTIBODY TITRES ($Log_{10}SN_{50}$) IN CATTLE INFECTED WITH RBKO VIRUS

	Days post-inoculation		
Animal No.	3	6	9
Z32	<0.3	2.6	3.8
Z36	<0.3	NT	NT
Z37	<0.3	2.2	NT
Z44	<0.3	2.2	NT
Z49	<0.3	NT	NT
Z51	<0.3	2.5	3.4
Z54	<0.3	NT	NT
Z56	<0.3	1.8	NT

NT = Not tested

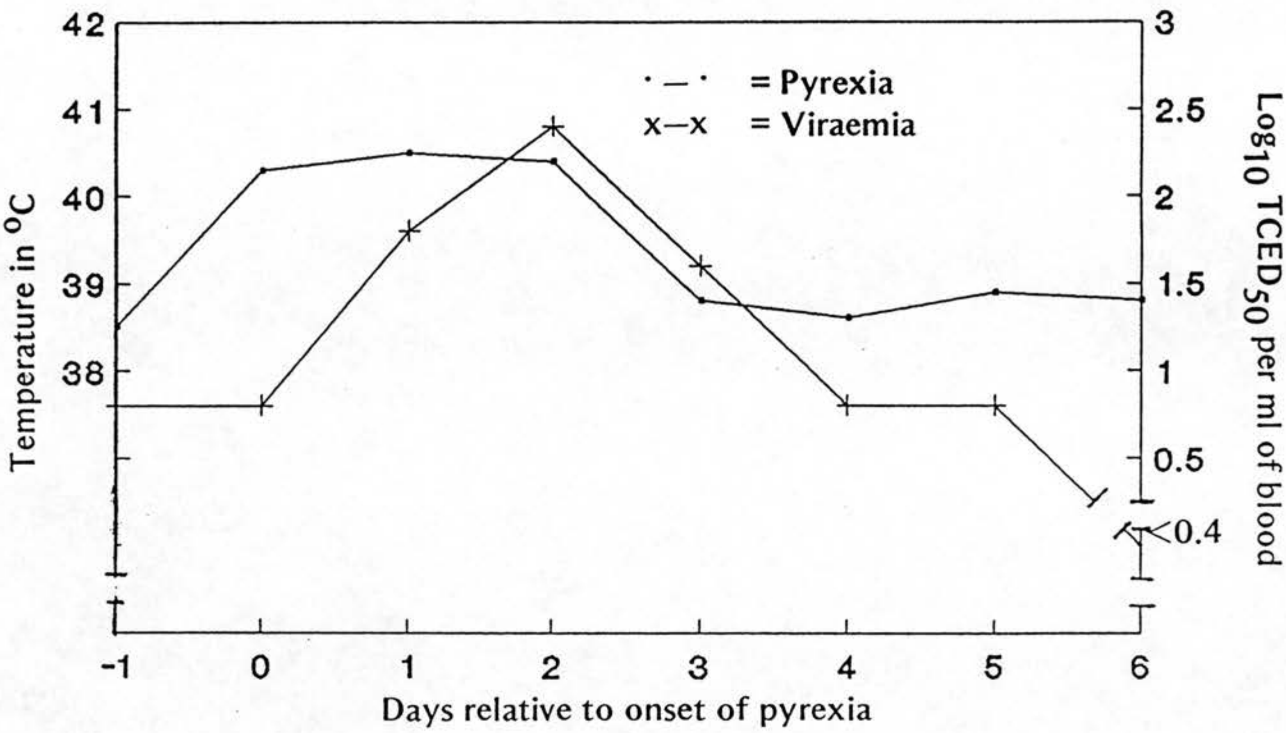

FIGURE 3.1 CORRELATION BETWEEN PYREXIA AND VIRAEMIA IN CATTLE INFECTED WITH RBKO VIRUS

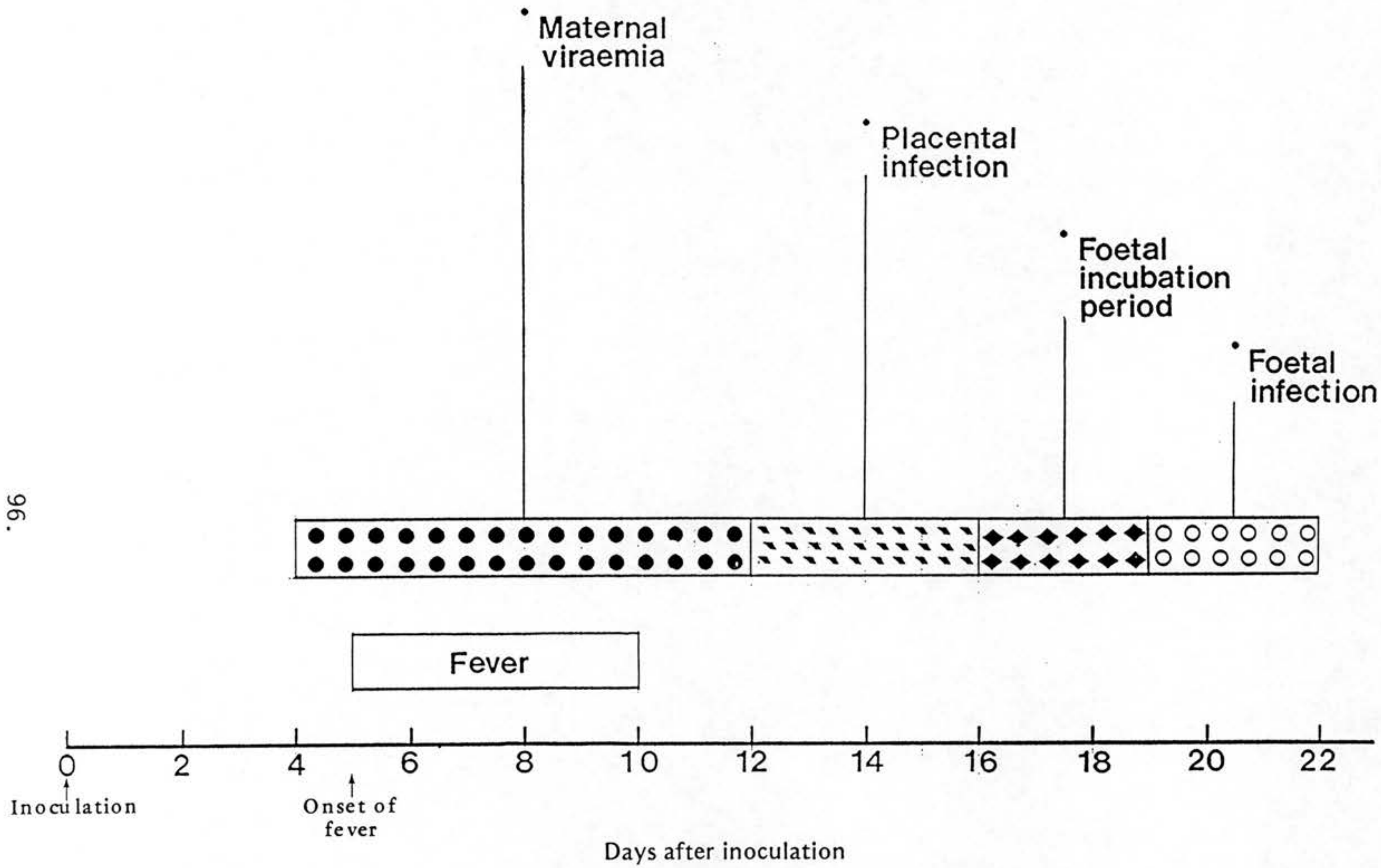

FIGURE 3.2 SPECULATIVE PATHOGENESIS OF RBKK VIRUS INFECTION IN COW Z68 AND HER FOETUS

CHAPTER FOUR

ATTEMPTED TRANSMISSION OF RINDERPEST VIRUS FROM ABORTING TO SUSCEPTIBLE CATTLE

INTRODUCTION

Natural rinderpest infections in cattle are contracted through direct contact between sick and healthy animals (Ramazzini, 1711), the infectivity being acquired from aerosols liberated by live sick animals (Hornby, 1926, Idnani, 1944; Liess and Plowright, 1964). However, pigs have been observed to become infected by eating infected carcasses (Molinie, 1931), and this has been confirmed by Scott, De Tray and White (1959). Scott (1976) has reported successful immunization of cattle with lyophilized caprinized rinderpest vaccine by oral administration in saline, fed in bran or offered as salt. It has, in addition, been asserted that pregnant cows which recover from rinderpest infection abort and that vaginal discharges and foetal tissues from such aborting cows are infectious on parenteral inoculation into susceptible cattle (Jacotot, 1931). Transmission of infection by contact with aborting cattle has not been reported.

The Second Chapter of this thesis records a number of abortions which occurred in cattle following recovery from experimental rinderpest and provides evidence of the presence of virus and virus antigens in maternal vaginal discharges and aborted foetal tissues. The obvious sequel therefore, was to carry out experiments to determine whether or not virus could be transmitted by contact between aborting and susceptible cattle. Unequivocal transmission did not occur but the data are herein provided.

MATERIAL AND METHODS

Cell cultures

BK cell cultures were used after preparation as previously described (Chapter Two).

Virus strain

The 2nd BK-passage RBKK virus was used at an infectivity titre of $10^{2.2}TCED_{50}$ per ml.

Animal inoculation

Nine adult Zebu cows between 3- and 7-months pregnant were each inoculated subcutaneously with 2 ml of the RBKK virus suspension. The animals were housed in isolation quarters and provided with hay and water *ad libitum*. They were examined four times daily for clinical signs of disease. At the onset of signs of abortion each cow was immediately moved into a separate clean room in close contact with 2 rinderpest susceptible, 1-2-year-old grade steers. Aborted foetuses and foetal membranes were left on the floor in the rooms for

24 hours before being removed and incinerated. Contact between the aborting animals and the steers was maintained for a further 4 weeks, the animals being examined twice a day for clinical signs of rinderpest.

Collection and processing of samples

Blood samples for serum antibody assays were collected at weekly intervals for 4 weeks following inoculation or contact exposure. Blood in EDTA was collected daily for two weeks after inoculation or following onset of pyrexia in in-contact cattle and again daily for one week following abortion. Vaginal swabs were collected from the cows daily until two weeks after abortion. Where possible, foetal blood in EDTA and for serum together with foetal membranes, tissues and fluids were collected as soon as practicable after abortion. Leucocyte fractions were prepared and examined for the presence of virus as previously described (Chapter Two).

Swab extracts and foetal fluids and tissues were assayed for virus infectivity, the latter two also being screened for the presence of rinderpest virus antigen. Tests for the presence of neutralizing antibodies were carried out on maternal and foetal sera and also on foetal tissue exudates and abdominal and thoracic fluids. Sera collected at the time of abortion were also tested for antibodies to BVD virus and *B. abortus*.

RESULTS

Clinical signs in pregnant donor cows

Four out of the 9 cows developed a rise in temperature of above 39.5^{o}C on the 5th day after inoculation and the remaining 5 cows reacted the following day. The duration of fever was 3 days in four cows, 4 days in three cows and 6 and 8 days in the remaining two cows (Table 4.1). The median incubation periods and durations of fever were thus 5 and 4 days respectively.

Mouth lesions consisting of focal necrosis and erosions of the epithelia occurred in all animals from the 8th to 10th days after inoculation i.e. the 3rd to 5th day after the onset of fever

Diarrhoea was somewhat delayed and occurred in only 5 cows from the 9th and 10th day after inoculation i.e. 5 to 6 days after the onset of fever (Table 4.1).

Two animals, B4 and B5, died on the 13th and 21st days after infection i.e. 8 and 15 days after the onset of fever (Table 4.1). A dead 8-month-old foetus was recovered post-mortem from cow B4. Cow B5 aborted a 3.5-month-old foetus on the 14th day after inoculation i.e. on the 2nd day of the remission of fever and 7 days before she died. Cows B1, B7 and B8 aborted 5-, 5- and 6-month-old foetuses on the 23rd, 5th and 6th days after the remission of fever respectively (Table 4.2) and made progressive recoveries thereafter. Cow B9 delivered a live, weak calf on day 119 following recovery. The calf died within an hour of delivery. Cows B2, B3 and B6 also gave birth to live calves 130, 162 and 149 days after recovery (Table 4.2).

The majority of the cows that survived infection with this strain generally showed recovery from the clinical disease 2-3 days after the remission of fever.

Virus isolation from pregnant donor cows

Rinderpest virus was first detected in the blood of four cows one day before the onset of fever (Table 4.3). All cows were viraemic at the onset of fever. Peak viraemias ranged from $10^{1.2}TCED_{50}$ per ml in cow B8 to $10^{2.2}TCED_{50}$ per ml in cows B1 to B4. The duration of viraemia ranged from 5 to 8 days with a median of 7 days.

Rinderpest virus was recovered from vaginal secretions of the 9 cows beginning on the day of onset of fever. The peak virus titre in the vaginal secretions averaged $10^{2.2}TCED_{50}$ per swab attained on the 5th day following the onset of fever and the duration of virus secretion through the vagina was 5 to 8 days.

Rinderpest virus was not demonstrated in foetal thymus, spleen, mesenteric lymph nodes, cotyledons and fluids from any aborted foetuses or live born calves.

Antigen detection in aborted foetuses

Rinderpest virus antigens were not demonstrated in any aborted foetal tissues and fluids.

Antibody detection

Low levels of neutralizing antibodies to rinderpest virus were first detected in the dams on the 7th day after inoculation and high titres were attained by the 14th day after inoculation (Table 4.4).

Neutralizing antibodies were not detected in aborted foetal sera and tissue exudates. Antibodies to BVDV and B. abortus were not detected in aborting cattle sera.

Pre-colostrum antibody titres ranging from 2.6 to 3.9 $\log_{10}$ SN_{50} were demonstrated in the calves born to cows B2, B3, B6 and B9 (Table 4.2). The cows had serum antibody titres of 3.0 to 4.0 $\log_{10}$ SN_{50} at the time of delivery.

Clinical signs in contact cattle

The 2 rinderpest susceptible grade steers kept in contact with cow No. B5 reacted with temperature elevation 8 and 10 days later. Peak pyrexia of 40.5^{o}C was attained on the 2nd day of fever and the duration of fever was 4 days in one animal and 8 days in the other.

Fever in both animals was accompanied by congestion and hyperaemia of the visible mucosal surfaces. Serous nasal and ocular discharges were observed for 3 and 6 days respectively. Oral lesions in the form of focal necrosis and erosions on the gums, lips, papillae tips and lower surface of the tongue developed in the in-contact cattle beginning from the 4th to the 6th days following the onset of fever. Watery diarrhoea which lasted 3 and 5 days developed in these steers.

No clinical disease suggestive of rinderpest was observed in the steers kept in contact with the 3 other cows that aborted nor in those in contact with the 4 cows, B2, B3, B6 and B9 that gave birth to normal calves.

Virus isolation from in-contact cattle

Rinderpest virus was demonstrated in the blood of the 2 steers kept in contact with cow B5 beginning from the day of onset of fever to one day following the remission of fever. Titration for virus infectivity levels in the blood of these animals was not conducted. Rinderpest virus was not detected in the blood of the remaining in-contact steers.

Antibody development in in-contact cattle

Antibodies to rinderpest virus were detected in the two steers kept in contact with cow B5 on the 14th day following the onset of fever in the steers. Specific antibodies to rinderpest virus were not detected in the other non-reacting steers.

Foetal pathology

The 5-month-old foetus expelled by cow B1 on the 23rd day after the cessation of fever appeared congested and grossly oedematous. The other foetuses appeared normal but had an accumulation of blood stained abdominal and thoracic fluids. Foetal cotyledons from aborted foetuses were discoloured yellowish-brown.

DISCUSSION

Sustained attempts to produce cases of rinderpest in susceptible steers by a 24-hour contact exposure to aborted and aborting cattle proved largely unsuccessful. With the exception of the steers exposed to cow B5 that aborted 2 days following the cessation of fever, there was no clinical, virological or serological evidence of transfer of infection from the aborted foetuses and their dams to in-contact susceptible cattle. Cow B5 aborted 2 days after the remission of fever i.e. soon after the acute stage of the disease. There was no demonstrable virus in the foetus, foetal membranes and fluids nor in maternal vaginal discharges at the time of abortion. This would suggest that the in-contact steers were infected by the dam and not the aborted foetus or foetal afterbirths. However, the abortion occurred 4 days after the regression of viraemia in the dam. BUT there is no evidence that cattle can secrete virus 4 days after the regression of viraemia. Most studies show a disappearance of infectivity from infected animal tissues 1-2 days after the regression of viraemia (Liess and Plowright, 1964; Plowright, 1964; Taylor et al, 1965). It would thus be reasonable to assume that the 2 steers in contact with cow B5 acquired infection from the aborted foetus and foetal discharges. The failure to demonstrate infectivity in the foetus using BK cells was possibly due to low susceptibility of the cells to the RBKK virus isolate.

In their studies on the pathogenesis of rinderpest in cattle following contact exposure, Taylor et al (1965) found it "more difficult than expected to produce regular cases of rinderpest in cattle by 24-hour contact exposure to single live donor animals."

Although rinderpest can be transmitted easily to experimental cattle by many parenteral routes (Hornby, 1926; Hall, 1933; Henning, 1949; Liess and Plowright, 1964; Plowright, 1964) the outcome and severity of infection varies with different strains (Scott, 1959c) and the dose of the virus (Woolley, 1906). The quantity of virus escaping in secretions and excretions of an infected animal may vary considerably from animal to animal and would most probably depend on the strain of virus. Thus Taylor and others (1965) failed to demonstrate virus infectivity in 20 of the 35 animals tested after housing them for 24 hours in stalls containing rinderpest infected animals which had been reacting to the disease for 3-5 days.

In addition a comparable irregularity of contact transmission with some Indian strains of rinderpest virus was reported by Cooper (1932) who found that exposure periods of 10 days sometimes failed to transfer infection to susceptible cattle, while other animals in continuous contact with presumed virus excreters did not react for 31-33 days.

Although it has been stated that rinderpest-infected carcasses are capable of transmitting infection by contact with susceptible animals (DeLay _et al_, 1962) and that vaginal discharges from cattle which abort following recovery from rinderpest are infectious on parenteral administration to susceptible cattle (Jacotot, 1931), I am not aware of any studies undertaken to provide quantitative data on the regularity of transfer of infection from dead and aborting animals to susceptible in-contact cattle.

Taylor and his colleagues (1965) using a highly virulent strain found it difficult to transmit the disease from sick to healty in-contact animals. Contact transmission of infection from dead foetuses and aborting cows exposed to a comparatively low virulent virus isolate such as the RBKK would therefore be expected to be even more difficult.

The possibility of transmission of rinderpest virus infection from aborting to susceptible cattle by contact cannot be dismissed entirely on the basis of the results obtained in this study. Only 4 of the 9 infected cows aborted and only one of these aborted more than one week after recovery. This is too small a number to show conclusively that such transmission cannot occur. More studies employing a large number of pregnant animals and different strains of rinderpest virus are required to elucidate this important aspect of the pathogenesis of the disease.

TABLE 4.1 CLINICAL RESPONSES OF CATTLE INFECTED WITH RBKK VIRUS

Cow No.	Incubation period (days)	Days of fever	Mouth lesions	Diarrhoea	Deaths
B1	4	4	+	-	-
B2	5	3	+	+	-
B3	5	6	+	+	-
B4	5	3	+	+	+(8)
B5	4	8	+	-	+(15)
B6	4	4	+	+	-
B7	5	3	+	-	-
B8	4	3	+	+	-
B9	5	4	+	-	-

( ) = Day the animal died after the onset of fever

TABLE 4.2 FOETAL INFECTION AND ABORTION IN CATTLE INFECTED WITH RBKK VIRUS

Cow No.	Abortion	*Day of abortion	*Day of normal delivery	Foetal antibody $Log_{10}SN_{50}/ml$	Contact transmission
B1	+	23		<0.3	-
B2	-		130	3.6	-
B3	-		162	3.9	-
B4—	-			<0.3	-
B5	+	2		<0.3	+
B6	-		149	2.6	-
B7	+	5		<0.3	-
B8	+	6		<0.3	-
B9	-		119	3.0	-

* = After remission of fever

— = Died when pregnant

TABLE 4.3 VIRAEMIA ($Log_{10}TCED_{50}$ per ml of blood) IN CATTLE INFECTED WITH RBKK VIRUS

	ANIMAL NUMBERS								
Days*	B1	B2	B3	B4	B5	B6	B7	B8	B9
-2	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4
-1	>0.4	<0.4	<0.4	>0.4	<0.4	<0.4	<0.4	>0.4	>0.4
0	>0.4	>0.4	>0.4	>0.4	>0.4	>0.4	>0.4	>0.4	>0.4
1	1.2	0.6	0.6	1.4	1.0	0.6	0.8	0.8	1.0
2	2.0	1.6	2.2	2.2	1.6	1.4	1.2	1.2	0.8
3	2.2	2.2	2.0	2.2	1.6	2.0	1.6	0.8	1.4
4	>0.4	>0.4	1.8	2.0	1.8	>0.4	1.0	1.0	1.8
5	>0.4	0.8	>0.4	1.4	0.8	>0.4	<0.4	<0.4	>0.4
6	>0.4	<0.4	<0.4	>0.4	>0.4	>0.4	<0.4	<0.4	<0.4
7	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4

* = Relative to onset of pyrexia

TABLE 4.4 ANTIBODY TITRES ($Log_{10}SN_{50}$) IN CATTLE INFECTED WITH RBKK VIRUS

Cow No.	DAYS AFTER INOCULATION 7	14
B1	2.2	3.6
B2	2.2	4.2
B3	1.9	3.8
B4	2.5	3.8
B5	1.9	3.6
B6	2.8	4.0
B7	2.1	3.3
B8	1.9	3.2
B9	1.9	3.8

CHAPTER FIVE

ABORTION IN CATTLE AS A SEQUEL TO NATURAL INFECTION WITH RINDERPEST VIRUS

INTRODUCTION

Rinderpest continues to occur in East Africa despite recent efforts to control the disease by widespread vaccination in the region (Rossiter *et al*, 1987). In Kenya rinderpest was, for the first time after an interlude of over ten years, reported in 1986 in the Pokot district of Western Kenya (Wafula and Kariuki, 1987). Since then two more outbreaks have been encountered, one in the Marsabit area of Northern Kenya in 1987 and the latest in the Kajiado, Kiambu and Nairobi districts of Central Kenya in August to September 1988 (Wamwayi and others, 1989). Because of my interest in rinderpest-induced abortion, the opportunity was taken to monitor the incidence of abortion during and after the outbreak of the disease in Central Kenya. The results are herein recorded.

BACKGROUND

With the exception of a few unconfirmed sporadic outbreaks of rinderpest along the northern and western borders, Kenya was virtually free from the disease by the end of the International Rinderpest Vaccination Programme popularly known as JP15 in 1976 (Provost, 1979).

The prolonged and severe drought which swept through the whole of the subtropical region of Africa in 1984 claimed many victims. In

Kenya alone, 4.5 million head of cattle and thousands of sheep and goats were estimated to have died. Animals most affected were those in the arid areas of the semi-nomadic Maasai and the traditionally similar tribes in the north, northwest and eastern parts of the country. The heavy losses led to an acute shortage of meat and meat products in the country particularly in the central cities such as Nairobi. Consequently, massive uncontrolled movements of livestock occurred, not only from the various corners of the country but more so from neighbouring states, in an attempt to supply the demand for slaughter cattle and herd replacements. The result was a collection of a considerably large number of cattle of uncertain vaccination history in and around Nairobi and the nearby Kajiado and Kiambu districts (Figure 5.1). It was against this background that a disease resembling rinderpest was first reported in February 1988 in western Kiambu district.

HISTORY OF THE OUTBREAK

The outbreak was first reported in a herd of 20 cattle on a farm in the Lusigeti area. Lusigeti shares a common boundary with Kibiko stock market in Kajiado district and is very close to Nguirubi grazing area of Kiambu district (Figure 5.2). Before the outbreak was reported, the 20 animals involved were herded on the large grazing area at Nguirubi where they came into contact with market stock recently purchased in northern Kenya and moved illegally to the Kibiko market in nearby Kajiado district. The disease was observed in 12 of the 20 animals. One adult and 4 calves died.

In April another outbreak of rinderpest-like disease occurred in a herd of slaughter cattle at the Dandora slaughterhouse in Njiru area of eastern Nairobi. A few cattle had previously been moved from the Kibiko stock market to Dandora and out of a herd of 90 adult Zebu cattle 30 died from the disease.

In July the disease was reported in a large herd of cattle on Waunyomu Ngeke Ranch in Ruiru area of eastern Kiambu. The history of animal movements indicated that cattle had recently been introduced into the herd from the Njiru area of Nairobi. The disease persisted on the ranch up to September 1988 by which time a total of 250 head of cattle had been affected and 90 had died.

By August the disease had spread from Lusigeti in western Kiambu through Nachu, Kiriri, Nguirubi to neighbouring Ewaso Kedong area in Kajiado district (Figure 5.2). The Maasai in Ewaso Kedong reported 60 cattle deaths, mainly calves, from a herd of approximately 500 cattle in which over 350 had fallen sick. The Maasai reported the disease after it had been present in their area for over a month and they claimed their animals had become infected following contact with cattle at Gicheru, a communal salt-lick and watering point near Nguirubi in Kiambu district.

Soon after the laboratory confirmation of the disease in these areas vaccination of cattle was immediately carried out in and around the affected areas using live attenuated tissue culture rinderpest vaccine (Plowright and Ferris, 1962). The disease subsequently disappeared from the affected herds about two weeks after vaccination.

MATERIALS AND METHODS

Collection and testing of samples

Samples for the confirmation of rinderpest were collected in every affected herd from at least 6 sick animals in the early stages of the disease, the sampling markers being the presence of fever and mouth lesions. Animals with diarrhoea were not sampled. Conjunctival sacs, upper respiratory tract and the mouth of each animal were swabbed using 2 swabs. One swab was immersed in 3 ml BAPBS for virus isolation and the contents of the second swab expressed and used undiluted for rinderpest virus antigen detection in the AGID test. In addition, aspiration biopsies were taken from prescapular lymph nodes and tested undiluted for rinderpest virus antigen.

Blood in EDTA and also without the anticoagulant was collected in sterile 25 ml bottles or in vacutainer tubes. In one instance, a volume of 50 ml of blood in EDTA was collected from a clinically sick cow in Nguirubi area of Kiambu district. Two pregnant Zebu cows were each inoculated intravenously with 20 ml of the blood. The clinical and virological responses of the 2 pregnant cows to inoculation with the 20 ml of blood have been discussed (Chapter Two). A serial 10-fold dilution of the buffy coat prepared from the remaining 10 ml of blood was made in PBS and inoculated onto BK cell monolayers in culture tubes using 5 tubes per dilution. The inoculated cultures were incubated at 37^{o}C overnight. The inoculum was then discarded, the cultures washed twice with warm PBS and overlaid with fresh ES maintenance median supplemented with 2 per cent ox serum. The cultures were again incubated at 37^{o}C and examined every 3 days for the presence of virus CPE. EDTA blood samples in vacutainer tubes were similarly assayed for virus presence.

Outbreak of abortion in cattle

From mid-September 1988 reports of abortion in several cows that recovered from the disease and had been vaccinated were received from Ewaso Kedong in Kajiado, Nguirubi in Kiambu and Waunyomu Ngeke ranch in Ruiru. Not all herds in these areas were infected with rinderpest but all were vaccinated. Hence all the vaccinated herds in these areas were visited and examined for evidence of abortion. Stockowners and herdsmen were questioned regarding the number of aborting animals in proportion to the number of affected pregnant cows, stages of gestation at the time of abortion, time of abortion relative to clinical recovery from the disease and evidence of retained afterbirths.

A dead foetus estimated from its crown-rump length of 40 cm and physical appearance (hairs on the muzzle) to be 5 months old was submitted to the laboratory from Nguirubi with a history of having been aborted by a cow that had recovered three weeks earlier from a natural attack of rinderpest. Gross pathological examination was carried out and foetal thymus, lung, spleen, liver and mesenteric lymph nodes tested for the presence of rinderpest virus and precipitating antigens.

Serology

Serum samples were collected from cows that had aborted following recovery from natural rinderpest. A total of 46 samples were collected, 4 from the herd in Ruiru and 42 from animals that had aborted in Nguirubi. The sera were tested for the presence of antibodies to rinderpest, BVD virus and B. abortus.

RESULTS

Clinical signs in the field

The disease occurred in areas where indigenous Zebu cattle were reared or kept and it affected cattle of all ages. The clinical signs frequently observed in the affected animals were fever 40.5^{o}C, excessive lachrymation, anorexia, depression, seromucoid and mucopurulent ocular and nasal discharges, dry encrusted muzzle, necrotic papillae tips, and focal to diffuse erosions and necrosis on the gums, lower surface of the tongue and hard palate. The majority of the animals had a watery diarrhoea sometimes containing blood. A few had bilateral corneal opacity. The morbidity rate was about 80 per cent. Twenty to 40 per cent of the calves aged up to 18 months died while the mortality rate among the adult cattle was much lower ranging from 5-10 per cent. A total of 900-1,000 cattle are estimated to have been affected by the disease.

Information on the incidence and times of abortion in pregnant cattle following recovery from natural rinderpest was obtained from stockowners and herdsmen (Table 5.1). Affected and non-affected herds in each area were paired and particular attention paid to the number of cows at risk in affected herds, number clinically affected, number aborting and time of abortion following clinical recovery. From approximately 158 pregnant cows that were affected and recovered in Nguirubi area of Kiambu district, 51 aborted from the 2nd to the 4th week after recovery. In Ewaso Kedong area of Kajiado district 60 out of 180 pregnant cows aborted between the 2nd and 4th week following vaccination and disappearance of disease from the area. Six out of 48 pregnant cows from a previously affected herd of cattle in Ruiru Kiambu aborted during the outbreak and up to 4 weeks post vaccination and recovery. Several cows in Ewaso Kedong and Nguirubi were found with retained after births and information from herdsmen indicated that about 5 per cent of the cows that aborted retained their afterbirths. Approximately 470 pregnant cows from herds that were not affected by the disease in the above areas and had also been covered in the vaccination programme did not abort before or after the vaccination (Table 5.1).

Virus isolation and antigen detection from field samples

Although rinderpest was suspected in the herds examined in Lusigeti in February, Dandora in April and Ruiru in July, no virus or virus precipitating antigens were demonstrated in BK cells or AGID test respectively from all samples collected in these areas. Ocular and nasal swabs from 8 out of 15 samples from 6 animals examined in Ewaso Kedong in August were positive for rinderpest virus antigen in AGID tests (Table 5.2). One out of 7 ocular swabs and 3 out of 7 nasal swabs from cattle in Nachu, Kiriri and Nguirubi in Kiambu were positive for rinderpest virus antigen in August. In the Ruiru ranch 1 out of 6 nasal swabs had precipitating antigens in August.

Rinderpest virus was recovered in BK cells inoculated with blood from a sick cow found in Nguirubi, Kiambu. The virus fulfilled Koch's postulates as follows:-

Virus CPE was first observed in the monolayer cultures on the 14th day of incubation at $37^{o}C$. The virus titre was $10^{1.4}TCED_{50}$ per ml of blood. The isolate was identified by its specific neutralization with rinderpest hyperimmune serum. Tissue fluids from a pool of 5 tubes showing maximum CPE were harvested by one cycle of freezing and thawing and further inoculated onto 7- day-old secondary BK cells grown in 300 ml culture bottles. Virus CPE was evident in the cell sheet 8 days later. Culture fluids from affected bottles were harvested on the 12th day of inoculation when the cultures showed more than 80 per cent CPE.

Two rinderpest susceptible, 16- and 24-week-pregnant Zebu cows were each inoculated subcutaneously with 2 ml of the 2nd BK virus passage containing $10^{2.6}TCED_{50}$ per ml of virus suspension. Two rinderpest-susceptible grade steers, 1-2 years old were housed in close contact with the IV-inoculated cows from the day of inoculation (Figure 5.3).

The inoculated cows developed fevers of greater than $39.8^{o}C$ on the 4th and 5th days after inoculation (Table 5.3). The durations of fever were 4 and 6 days respectively. Oral lesions and slight diarrhoea developed in the cows from the 3rd and the 6th days following the onset of fever respectively. Viraemia was detected in one cow from the day preceding the onset of fever and from the day of fever in the other cow. Viraemia persisted in both cows up to the 5th and 6th days following the onset of fever (Figures 5.4a and b). Both cows aborted on the 5th and 17th days after recovery from the clinical disease and rinderpest virus antigen was demonstrated in the thymus and mesenteric lymph nodes obtained from the foetus aborted on the 17th day following the dam's clinical recovery from the disease. The two steers housed with the subcutaneously infected cows reacted with clinical signs characteristic of rinderpest from the 12th and 13th days of contact. Examination of the steers for viraemia on the 1st and 2nd days of fever revealed rinderpest virus.

Virus isolation and antigen detection in the foetus from the field

Rinderpest virus was not isolated in BK cells from thymus, spleen, lung, mesenteric lymph nodes and liver from the foetus aborted by the naturally infected cow in Nguirubi. Rinderpest virus antigens were however detected in the thymus and mesenteric lymph node from the same foetus.

Serology

All the 46 serum samples from the cows that aborted after recovery from natural rinderpest infection had a median rinderpest neutralizing antibody titre of 2.6 $\log_{10}SN_{50}$ per ml with a range of 1.8 to 3.6. The sera were negative for antibodies to Brucella abortus and bovine virus diarrhoea virus.

DISCUSSION

The occurrence of rinderpest in Nairobi and the nearby districts of Kajiado and Kiambu indicated that most of the cattle in these areas were susceptible to the disease. The initial attempts to confirm the disease were unsuccessful and this was probably due to the mild nature of the disease and the presence of low titres of virus in the tissues and secretions of the animals sampled, most of the animals having been sampled in the late stages of the disease because the presence of the disease on the farms was often reported late. Such factors have in the past been reported to hinder the rapid confirmation of rinderpest (White, 1958; Taylor, 1986).

The origin of the outbreak was not established but it is believed that the infection was probably introduced into Kibiko stock-market by live sick animals moved on motorised transport from Marsabit in Northern Kenya.

The epidemiological data recorded in this chapter add to the existing evidence incriminating rinderpest virus as a cause of bovine abortion (Layard, 1757; Nocard and Leclainche, 1896; Aldige, 1918). There is a scarcity of recorded information on bovine abortion in natural rinderpest outbreaks. This probably is because most of the classic descriptions of the clinical disease are based on virgin-soil epidemics in which highly susceptible animal populations have been affected the result being massive deaths in cattle of all ages including potentially abortive pregnant cows. Under these circumstances much attention has been focused on the severity of the disease and the ensuing heavy mortalities and little has been paid to abortion as a clinical feature of the disease (Ramazzini, 1711; Lancisi , 1715; Hall, 1966; Mack, 1970).

In contrast, hardly any descriptions exist on the clinicopathological features of rinderpest in endemic situations where low virulent strains of virus infect cattle with relative high innate resistance acquired through previous ancestral exposure (Scott, 1985). Under these conditions the disease occurs in a mild, often unrecognisable form affecting mainly young susceptible yearlings, most adults including pregnant ones being immune either from vaccination or previous natural infection. Very little attention has thus been paid to the clinical disease in endemic areas and no association has been made between the rinderpest infection and abortion. In Kenya for example, there are reports of numerous outbreaks of rinderpest and widespread abortions and sterility in cattle between 1927 and 1935 (Anon, 1927-1935). Although many farmers at that time maintained that the worst cases of sterility occurred in pregnant cattle that aborted following rinderpest infection, no effort was made to establish the relationship between the two conditions hence the farmers' observations were never confirmed (Anon, 1928).

In high risk areas adjacent to endemic situations there occurs a build-up of high susceptible cattle populations as a result of laxity of veterinary authorities and stockowners towards vaccinations. When animals in such situations become exposed to low virulent virus strains from adjacent endemic areas, high abortion rates similar to those recorded here are likely to occur. These situations are rare.

In the few reports that have been made associating bovine abortion and natural rinderpest infection (Layard, 1757; Aldige, 1918), no detailed laboratory investigations of the clinical episode and subsequent abortions were conducted. In the present outbreak a confirmatory diagnosis of rinderpest preceded the onset of abortion which occurred mainly in cows that had suffered and recovered 2 to 4 weeks previously. A high incidence of abortion such as the present one had not been encountered in the affected herds prior to the outbreak and because abortion as a possible sequel to natural rinderpest infection was not envisaged the stockowners were not alerted. Consequently its occurrence was immediately attributed to the vaccine employed. This opinion did not find support as there were no abortions in nearby herds that were not affected by the disease even though they also were vaccinated using the same culture-passaged rinderpest virus vaccine.

These observations confirmed the earlier findings by Plowright and Ferris (1962) that the culture-passaged rinderpest virus vaccine does not cause abortion in pregnant cows and was therefore, not the cause of abortions in the current epidemic.

It is evident that little epidemiological significance has been paid to abortions that occur in cattle following recovery from rinderpest infection in the field. Such animals may constitute a danger of transmitting infection to susceptible animals with which they come into contact at the time of abortion.

TABLE 5.1 ABORTION IN CATTLE NATURALLY INFECTED WITH RINDERPEST VIRUS

Area	Herd No.	No. of pregnant cows	No. of pregnant cows affected	NUMBER OF ABORTING COWS 1 month to outbreak	During outbreak	2-4 weeks after vaccination and recovery	Total abortions	Per cent abortion
Nguirubi	1	197	158	Nil	7	44	51	32.3
	2	120	Nil	Nil	Nil	Nil	Nil	
Ewaso Kedong	1	200	180	Nil	Nil	60	60	33.3
	2	100	Nil	Nil	Nil	Nil	Nil	
Waunyomu (Ruiru)	1	60	48	Nil	2	4	6	12.5
	2	250	Nil	Nil	Nil	Nil	Nil	

TABLE 5.2 CONFIRMATION OF RINDERPEST OUTBREAK BY AGID TEST AND VIRUS ISOLATION

Area	SAMPLES TESTED (NO. POSITIVE/NO. TESTED) EDTA* blood	Ocular+ swabs	Nasal+ swabs
Nguirubi	1/12	1/7	3/7
Lusigeti	0/8	0/8	0/10
Dandora	0/8	0/6	0/6
Ruiru	0/6	NT	1/6
Ewaso Kedong	NT	1/4	7/11

* = Virus isolation
+ = Detection of antigen
NT = Not tested

TABLE 5.3 FULFILMENT OF KOCH'S POSTULATES WITH RBKK VIRUS ISOLATE

	ANIMAL NUMBERS			
	Y15	Y57	A228	A234
Route of inoculation	SC	SC	C	C
Incubation period (in days)	3	4	8	9
Days of fever	4	6	4	6
Mouth lesions	+(6)	+(2)	+(4)	+(4)
Diarrhoea	+(3)	+(5)	+(4)	+(6)
Viraemia	+(7)	+(7)	+	+
Day* aborted	5	17	NA	NA

* = Days after clinical recovery
( ) = Number of days the feature was observed
NA = Not applicable
C = Contact with Y15 and Y57

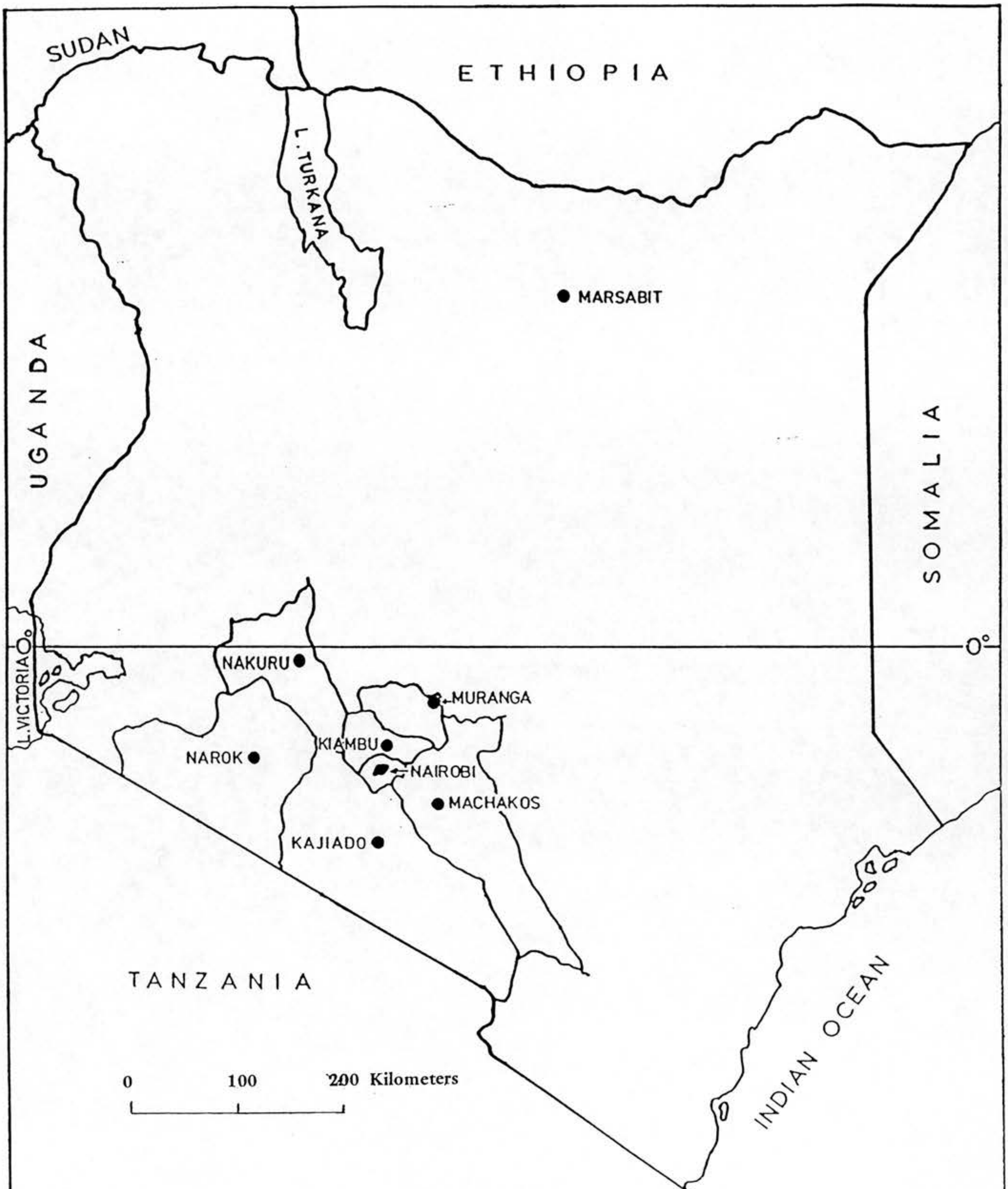

FIGURE 5.1 MAP OF KENYA SHOWING SOME DISTRICTS AFFECTED BY A RINDERPEST OUTBREAK IN 1987–1988

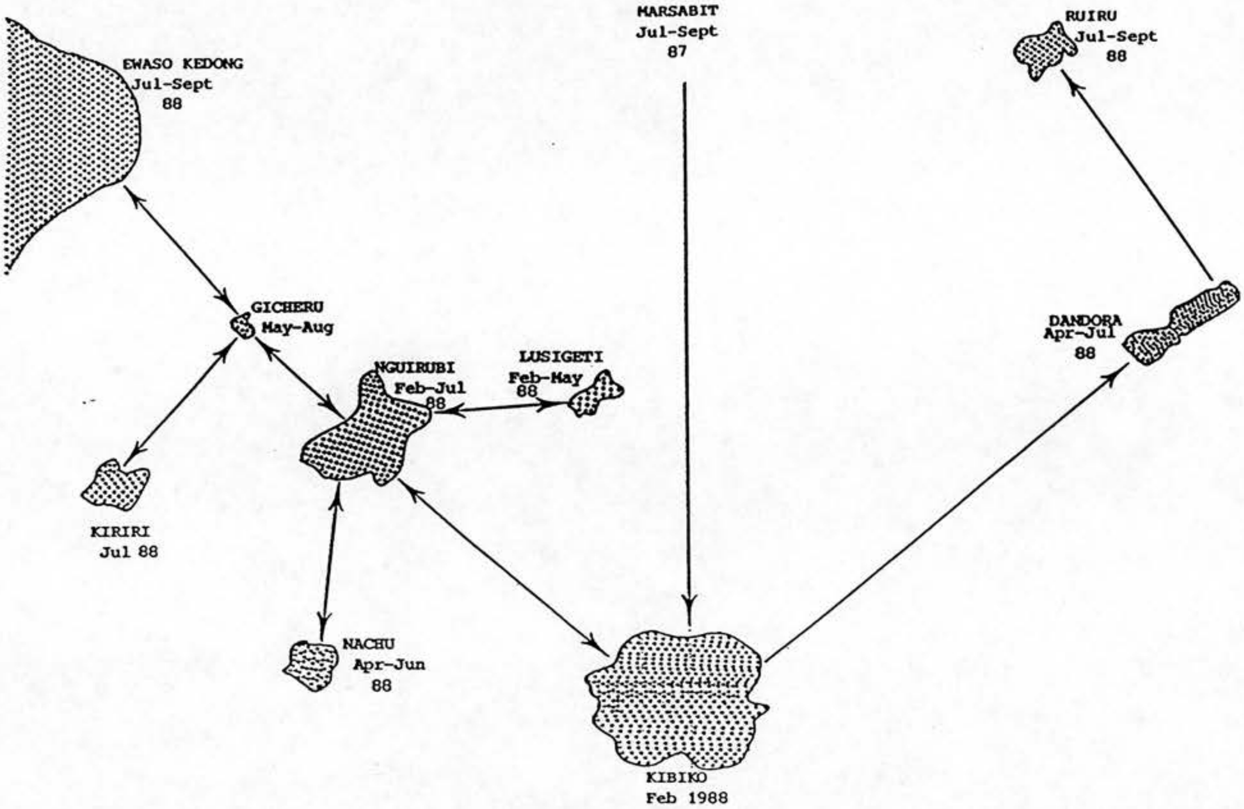

FIGURE 5.2 SPREAD OF RINDERPEST IN KENYA, 1987–1988

Sick cow in the field **(1)**

↓

Blood in EDTA

↓

Leucocyte fraction

↓

BK cell cultures

↓

Cytopathic agent → Neutralized by rinderpest hyper-immune serum

↓

Further inoculation on BK cell cultures

↓

Cytopathic agent

↓

2 steers in contact → Inoculation of 2 pregnant cows

2 steers in contact	Inoculation of 2 pregnant cows	
↓	↓	
Clinical disease identical to that in (1)	Clinical disease identical to that in (1)	
↓	↓	
Buffy coat from EDTA blood	Buffy coat from EDTA blood	
↓	↓	
BK cell cultures	BK cell cultures	
↓	↓	
Cytopathic agent	Cytopathic agent →	Neutralized by rinderpest hyper-immune serum

FIGURE 5.3 FULFILMENT OF KOCH'S POSTULATES WITH RBKK VIRUS ISOLATE

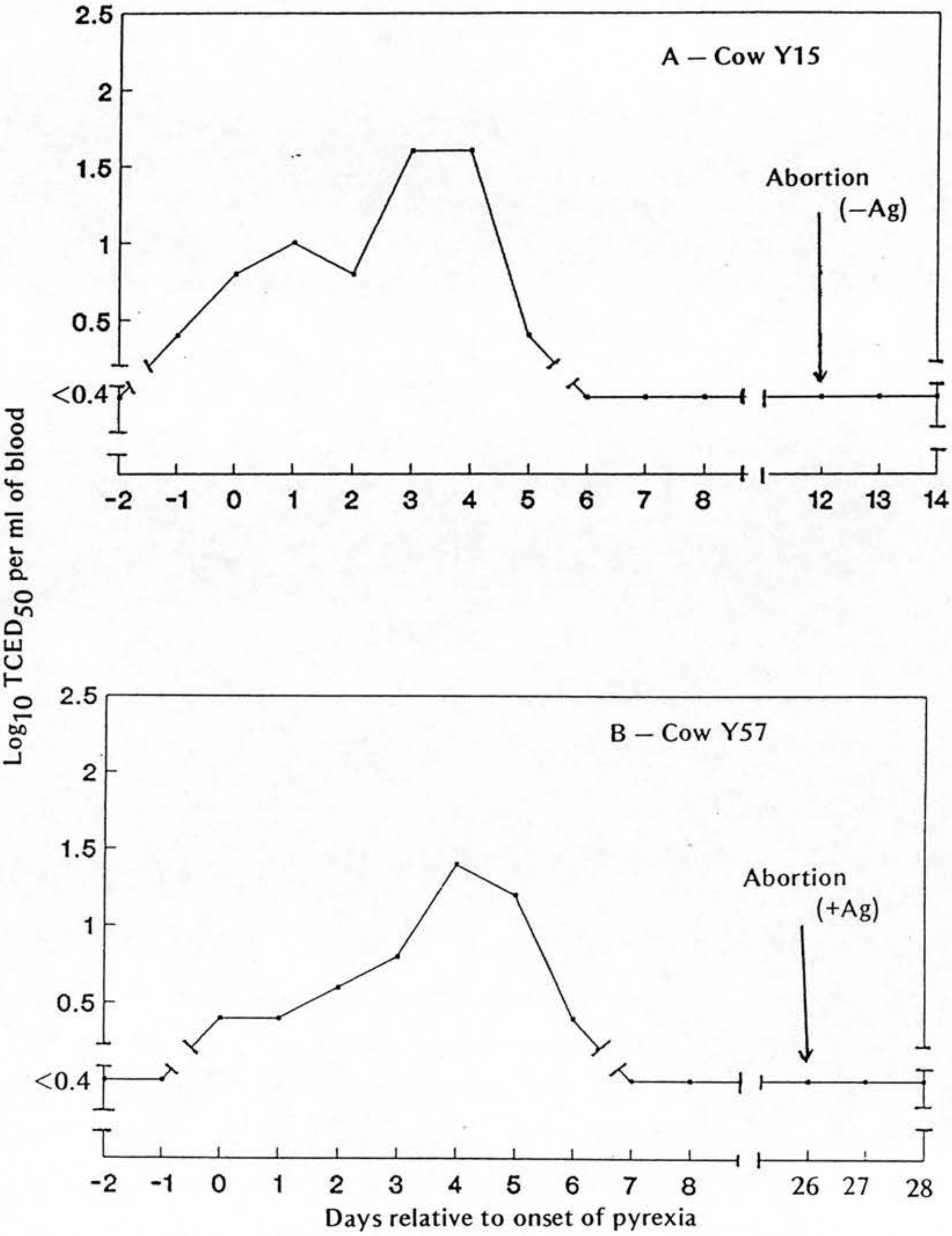

FIGURE 5.4 CORRELATION BETWEEN VIRAEMIA AND ABORTION IN CATTLE INFECTED WITH RBKK VIRUS

CHAPTER SIX

RESPONSES OF PREGNANT GOATS TO INFECTION WITH DIFFERENT STRAINS OF RINDERPEST VIRUS

INTRODUCTION

Ever since Edwards demonstrated the transmission of rinderpest virus from an inoculated caprine foetus to maternal blood in 1927, there have occurred a number of conflicting reports regarding trans-placental transmission of rinderpest virus in pregnant goats. Thus Banerji and Mohan (1935) while working at Mukteswar in India observed abortion in a goat one week after inoculation with a strain of the "goat-adapted" rinderpest virus. A pool of spleen and blood collected from the aborted foetus induced rinderpest when inoculated into 2 susceptible goats. In contrast, Cornell (1936) in Tanganyika and Sreenivasan (1942) in India could not induce disease in an ox or hill bulls following inoculation with homogenised foetal tissues and blood from caprine foetuses aborted or obtained from pregnant goats after infection with the "goat-adapted" rinderpest virus.

The artificial attenuation of rinderpest virus to goats revolutionized the control of rinderpest in buffaloes and cattle. Surprisingly, however, the use of goats as a model host in rinderpest research has been neglected. Their potential is great and it is in this context that the response of pregnant goats to infection with two strains of rinderpest virus was re-examined.

MATERIALS AND METHODS

Cell cultures

Bovine kidney cells

The methods of preparation, growth and maintenance of bovine calf kidney (BK) cell cultures were as described earlier (Chapter Two).

Goat kidney cells

Primary goat kidney (GK) cell cultures were prepared by the method of trypsinization in a manner identical to that described for BK cells.

Vero cells

Vero cells were purchased from Flow Laboratories, UK and were maintained by continuous passage in MEM medium supplemented with 10 and 2 per cent foetal bovine serum for growth and maintenance respectively plus antibiotics.

Virus strains

The Kabete attenuated goat (KAG) strain of caprinized rinderpest virus, moderately attenuated for cattle but highly virulent for goats (Daubney, 1949) and the RBKO strain, highly virulent for cattle and expected to be avirulent in goats were used to infect pregnant goats. The high cell culture-attenuated vaccine strain of rinderpest virus was used in serological tests.

Goats

The animals used were the indigenous Small East African goats (Devendra and Burns, 1983) (Figure 6.1). All were approximately 2 to 3 years old weighing 25-30 kg. They were mated to a buck of the same breed and the mating dates were recorded. The goats were screened before mating and again before inoculation and were found to be free of rinderpest neutralizing antibody (Rossiter and Jesset, 1982). The animals were accommodated in isolation units which precluded the possibility of accidental infection with rinderpest virus and were fed with hay and provided with water *ad libitum*.

Animal inoculation

Infection with the KAG virus

A group of 8 goats between 50 and 67 days pregnant was inoculated subcutaneously with 2 ml of a 20 per cent spleen suspension obtained on the 2nd day of fever from a KAG virus infected goat. A second group of 5 goats was similarly inoculated at approximately 110 days gestation (Table 6.1).

Infection with the RBKO virus

A third group of 3 goats between 50 and 65 days pregnant was injected subcutaneously with 0.5 ml of a reconstituted suspension of freeze-dried RBKO-infected cattle spleen. The goat inoculum was simultaneously titrated in BK cells and found to contain a titre of $10^{3.8}TCED_{50}$ per ml. A fourth group of 3 goats was similarly infected when approximately 110 days pregnant (Table 6.4).

Control animals

A total of 10 pregnant goats, 5 of them between 50 and 67 days pregnant and 5 at approximately 110 days pregnant were left as non-infected controls. They were housed separately in clean isolation units.

Rectal temperatures were recorded every morning and detailed clinical examinations performed daily.

Collection and processing of samples

Samples for virus isolation, agar-gel immunodiffusion and serology were collected from the does and aborted foetuses and were processed and tested as described earlier (Chapter Two). Virus isolation attempts were carried out by simultaneous inoculation of samples in BK, GK and Vero cell cultures and observed for 21 days for the development of viral CPE.

Two ml of 20 per cent suspensions of foetal thymus, spleen and mesenteric lymph nodes from aborted foetuses were in addition inoculated subcutaneously into rinderpest susceptible goats. The animals were examined for clinical and serological evidence of infection with rinderpest virus.

RESULTS

Goats infected with the KAG virus

Clinical signs

The incubation period in the 13 goats inoculated with the KAG virus varied from 2 to 6 days but the majority of the animals (7/13) showed a temperature reaction of greater than 39.5^{o}C on the 4th day (Table 6.1). Peak pyrexia was attained on the 2nd day following the onset of fever and the duration of fever was 1 to 4 days with a median of 3 days in both groups. The onset of fever was usually accompanied by an increase in respiration, anorexia and staring rough hair coat. Affected goats stood depressed with arched backs, and strained frequently 2-3 days later. This attitude often persisted till death or remission of fever. Mucoid nasal and ocular discharges developed in 8 out of the 13 goats from the 2nd day of fever and persisted up to 1 to 3 days after the remission of fever.

Animals did not develop definite oral lesions but hyperaemia and congestion of the oral and conjunctival mucosae accompanied the rise in temperature. Development of a watery diarrhoea occurred between the 2nd and 5th days following the onset of fever but half of the goats developed diarrhoea on the 4th day following the onset of fever (Table 6.1). The duration of diarrhoea was 5 to 8 days in goats that survived from the disease. Five out of the 8 goats inoculated at 50-67 days gestation and all the 5 goats inoculated between 106 and 110 days pregnant aborted. The median days to abortion were 4 and 7 respectively (Table 6.3) and the difference between these days was not significant (Mann Whitney $U = 3$, $P > 0.05$). Six goats died from the disease between the 5th and 11th days after the onset of fever (Table 6.1). The rest of the goats entered convalescence between 5 to 7 days after the remission of fever.

Virus isolation

Rinderpest virus was not isolated in BK, GK and Vero cells from any of the goats inoculated with the KAG strain of rinderpest virus. In addition, virus was not demonstrated in cultures inoculated with tissue homogenates and fluids from aborted foetuses.

There was no clinical, virological or serological evidence of rinderpest virus infection in the susceptible goats inoculated with tissue homogenates from aborted foetuses.

Antigen detection

Rinderpest virus antigen was detected in the spleen and mesenteric lymph nodes of goats G11, G18 and G2101 that died on the 5th and 6th days following the onset of fever (Table 6.1). Virus antigen was not demonstrated in similar tissues from goats G13, G32 and G2071 that died on the 8th, 10th and 11th day after the onset of fever respectively nor was it detected in all aborted foetal tissues that were examined.

Serology

The serological response of goats infected with the KAG-virus strain was tested on days 7, 14 and 21 post infection (Table 6.2). Low levels of virus neutralizing antibodies were demonstrated in most of the goats on the 7th day but titres above 2.0 $\log_{10}SN_{50}$ had developed in the survivors by the 2nd week of inoculation.

Pathology

The most prominent pathological signs noted at post-mortem examination of the KAG virus-infected goats were a slight interstitial pneumonia involving all the lobes of the lungs and a mild haemorrhagic abomasitis and enteritis. Four out of the 5 foetuses aborted following inoculation of the dams between 50 and 67 days gestation were macerated. The foetus from the fifth goat together with those expelled following infection of the dams at 110 days of pregnancy appeared grossly normal (Table 6.3).

Control animals

There was no clinical disease, foetal expulsion or development of neutralizing antibody to rinderpest virus in the 10 non-infected goats. These animals produced normal goat kids 145-150 days after mating.

Goats infected with the RBKO virus

Clinical signs

Two of the goats inoculated with the enhanced virulent RBKO virus strain developed fever of greater than 39.5^{o}C on the 3rd day of inoculation while the other 4 developed a rise in temperature on the 4th day. The median peak pyrexia was attained on the 2nd day and the duration of fever was 3 to 4 days (Table 6.4) with a median of 3 days.

The clinical signs were similar to those observed in the KAG virus-infected animals albeit more severe. The animals developed an ataxic posture towards the terminal stages of the disease that mainly affected the hind legs (Figure 6.2). Profuse bloody diarrhoea followed by prostration and recumbency were commonly observed 1 to 2 days before death. Two goats died on the 4th day and three on the 5th day following the onset of fever. Only one goat recovered clinically, 9 days after the onset of fever (Table 6.4). Two of the 3 goats infected at over 100 days pregnant aborted normal-looking foetuses on the 4th and 6th days after the onset of fever (Table 6.5). The foetuses from all the goats infected at 50-65 days and one from a goat infected when 110 days pregnant were found in situ and normal at post-mortem examination.

Virus isolation

Rinderpest virus was readily recovered in BK, GK and Vero cells from the blood and vaginal swabs of goats infected with the RBKO virus strain.

Virus was first detected in BK and Vero cells from the blood of one goat on the day preceding the onset of fever. The six goats were viraemic on the day of onset of fever (Table 6.6, Figure 6.3). The duration of viraemia was not determined as most of the animals died between the 4th and 5th days following the onset of fever when still viraemic. The duration of viraemia in one goat that survived was 9 days with peak titre of $10^{1.8}TCED_{50}$ per ml of blood being attained on the 3rd day of fever. Rinderpest virus was also detected in ocular and nasal secretions from the infected goats (Tables 6.7 and 6.8, Figures 6.4 and 6.5). Virus was first demonstrated in vaginal secretions from four goats on the 3rd day of fever. All goats were secreting virus via the vaginal tract by the 4th day of fever (Table 6.9 and Figure 6.6). One goat which recovered from the disease secreted virus through the vagina up to the 8th day following the onset of fever i.e. one day after the cessation of viraemia and 2 days after abortion. Rinderpest virus was recovered in BK cells from the placentomes of 3 infected goats (Table 6.5). The median virus titre in the placentomes was $10^{2.0}TCED_{50}$ per g of tissue. Virus was not recovered from the blood, tissues and fluids of any of the foetuses obtained after abortion or post-mortem.

Antigen detection

Rinderpest virus precipitating antigens were detected in maternal mesenteric lymph nodes of all goats that died but only in the placentomes from which infectious virus was isolated.

Serology

Only 1 of the 6 goats infected with the RBKO virus strain survived long enough for a meaningful serological assessment to be carried out and the pattern of its antibody development was similar to that in goats infected with the KAG virus strain (Table 6.10).

Pathology

In addition to pneumonia goats that died following infection with the RBKO virus strain had severe diffuse ulcers in the abomasum and small intestines and linear haemorrhages in the large intestines. A macerated foetus was recovered post-mortem from one of the goats infected on days 50 of gestation (Table 6.5). Normal-looking foetuses were aborted or recovered post-mortem from the other 5 goats.

DISCUSSION

Infection of pregnant goats with either the KAG or the enhanced RBKO strains of rinderpest virus produced a disease clinically similar to that previously described by Edwards (1927) and Beaton (1930). Unexpectedly, the disease was more severe and caused more deaths in the goats infected with the RBKO virus strain than in the animals infected with the goat-adapted virus. However, the difference in the morality rates was not significant (Fisher's exact test, P = 0.13); probably because of inadequate numbers of animals in the groups.

Infection of goats with the enhanced RBKO strain induced a readily detectable viraemia. The animals secreted virus through the tears, nasal and vaginal discharges. In contrast, virus could not be recovered in BK, GK and Vero cells from samples collected from the KAG virus infected goats. In comparing the host cell range of some attenuated vaccine strains of rinderpest virus, Plowright and Ferris (1959a) failed to propagate the goat adapted virus strain in BK and GK cells. More recently Guillemin, Jouvenet, Mosienyane and Mannathoko (1988) have remarked on the failure of caprinized rinderpest virus to produce CPE in BK cells but have not provided data on which they based their comments. There is no readily available information on the susceptibility of Vero cells to the growth of KAG strain of rinderpest virus. The present findings indicate that these cells are also refractory to the KAG strain.

Both strains induced abortion in pregnant goats when inoculated at 50-67 and 110 days. However, there was no virological evidence that infection of the goat foetuses by either virus had taken place although infectious virus was demonstrated in the placentomes from three goats inoculated with the RBKO virus strain. Our findings support the observations by Banerji and Mohan (1935) and Cornell (1936) that abortion may occur in goats infected with rinderpest virus but do not confirm reports of the demonstration of rinderpest virus in aborted caprine foetuses (Banerji and Mohan, 1935) or in foetuses obtained post-mortem from rinderpest-infected pregnant goats (Chawla and Sinha, 1961). The failure to demonstrate virus in the foetuses, particularly from goats infected with the RBKO strain, indicates that foetal infection had probably not taken place at the time of abortion or death, both of which occurred during or soon after the acute stage of the disease and possibly before transmission of the virus across the placenta to the foetus.

TABLE 6.1 CLINICAL RESPONSE OF GOATS INFECTED WITH THE KAG VIRUS

Animal No.	Gestation age (days) when infected	Incubation period (days)	Days of fever	Diarrhoea	Deaths or Recoveries
G10	67	4	2	+	11R
G11	50	2	3	+	6D
G18	60	5	4	+	5D
G28	50	4	3	+	10R
G31	63	4	2	+	7R
G32	65	4	3	+	10D
G2100	67	2	4	-	6R
G2101	67	3	1	+	6D
G13	110	4	4	+	8D
G14	108	4	4	+	7R
G23	106	6	3	+	5R
G46	110	3	2	+	5R
G2071	109	4	3	+	11D

D = Died on indicated day
R = Recovered on indicated day

TABLE 6.2 ANTIBODY TITRES ($Log_{10}SN_{50}$) IN GOATS INFECTED WITH THE KAG VIRUS

Animal No.	DAYS AFTER INOCULATION 7	14	21
G10	1.9	2.1	2.6
G11	1.9	NT	NT
G18	1.3	NT	NT
G28	1.3	2.0	2.3
G31	1.3	2.4	2.0
G32	1.6	2.6	NT
G2100	1.9	2.3	2.8
G2101	1.3	NT	NT
G13	1.3	NT	NT
G14	1.6	2.8	2.2
G23	1.6	2.0	2.5
G46	1.6	2.1	2.3
G2071	1.3	2.2	NT
Median	**1.6**	**2.2**	**2.3**

NT = Not tested

TABLE 6.3 ABORTION IN GOATS INFECTED WITH THE KAG VIRUS

Animal No.	Day* aborted	Foetal state	Evidence of foetal infection
G10	4	M	-
G11	NA	-	-
G18	NA	-	-
G28	1	N	-
G31	4	M	-
G32	3	M	-
G2100	8	M	-
G2101	NA	-	-
G13	7	N	-
G14	10	N	-
G23	4	N	-
G46	5	N	-
G2071	9	N	-

* = After the onset of fever
M = Macerated
N = Normal
NA = No abortion, foetus obtained post-mortem

TABLE 6.4 CLINICAL RESPONSE OF GOATS INFECTED WITH RBKO VIRUS

Animal No.	Gestation age (days) when infected	Incubation period (days)	Days of fever	Diarrhoea	Day* of death	Day* of recovery
G1	50	2	3	+	5	
G2	65	3	3	+	4	
G16	51	3	3	+	5	
G19	103	3	4	+	4	
G24	108	3	4	+	-	9
G29	110	2	3	+	5	

* = Following the onset of fever

TABLE 6.5 ABORTIONS IN GOATS INFECTED WITH THE RBKO VIRUS

Animal No.	Foetal state		Placentome infection*	Foetal infection
	in situ	aborted		
G1	M	-	-	-
G2	N	-	2.0	-
G16	N	-	1.8	-
G19	N	-	2.2	-
G24	-	N	-	-
G29	-	N	-	-

* = Virus titre in $Log_{10}TCED_{50}$ per g
M = Macerated
N = Normal looking

TABLE 6.6 VIRAEMIA IN PREGNANT GOATS INFECTED WITH RBKO VIRUS

Days relative to onset of fever	GOAT NUMBER					
	G1	G2	G16	G19	G24	G29
-1	<0.4*	<0.4	<0.4	<0.4	0.8	<0.4
0	0.8	0.4	1.0	1.0	1.2	0.8
1	1.4	1.2	1.8	1.4	1.6	1.4
2	1.8	NT	2.0	2.0	1.8	
3	2.0	2.0	NT	NT	1.8	1.8
4	1.4	NT	NT	NT	1.6	NT
5	NT	NT	NT	NT	0.8	NT
6	NT	NT	NT	NT	0.8	NT
7	NT	NT	NT	NT	1.4	NT
8	NT	NT	NT	NT	<0.4	NT

* = Virus titre in Log_{10} $TCED_{50}$/ml of blood

NT = Not tested

TABLE 6.7 OCULAR SECRETION OF VIRUS BY PREGNANT GOATS INFECTED WITH RBKO VIRUS

Days relative to onset of fever	ANIMAL NUMBER					
	G1	G2	G16	G19	G24	G29
-1	<0.4*	<0.4	<0.4	<0.4	<0.4	<0.4
0	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4
1	<1.4	1.6	<0.4	1.2	1.4	<0.4
2	1.4	2.0	1.6	1.6	1.2	1.6
3	1.8	2.2	2.6	2.2	1.6	2.0
4	NT	NT	1.6	NT	1.0	1.4
5	NT	NT	NT	NT	0.8	<0.4
6	NT	NT	NT	NT	<0.4	NT

* = Virus titre in Log_{10} $TCED_{50}$ per swab
NT = Not tested

TABLE 6.8 NASAL SECRETION OF VIRUS BY PREGNANT GOATS INFECTED WITH RBKO VIRUS

Days relative to onset of fever	ANIMAL NUMBER					
	G1	G2	G16	G19	G24	G29
0	<0.4*	<0.4	<0.4	<0.4	<0.4	<0.4
1	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4
2	1.0	1.4	2.0	<0.4	1.0	1.6
3	1.4	1.0	2.0	<0.4	1.6	1.6
4	1.0	1.2	1.6	<0.4	1.6	<0.4
5	NT	NT	NT	NT	0.8	<0.4
6	NT	NT	NT	NT	<0.4	NT

* = Virus titre in Log_{10} $TCED_{50}$ per swab
NT = Not tested

TABLE 6.9 VAGINAL SECRETION OF VIRUS BY PREGNANT GOATS INFECTED WITH RBKO VIRUS

Days relative to onset of fever	ANIMAL NUMBER					
	G1	G2	G16	G19	G24	G29
0	<0.4*	<0.4	<0.4	<0.4	<0.4	<0.4
1	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4
2	<0.4	0.4	1.4	0.8	0.4	<0.4
3	1.6	0.6	1.8	2.0	1.8	1.2
4	1.2	NT	1.4	NT	1.4	0.8
5	NT	NT	NT	NT	1.2	NT
6	NT	NT	NT	NT	1.2	NT
7	NT	NT	NT	NT	0.8	NT
8	NT	NT	NT	NT	0.8	NT
9	NT	NT	NT	NT	<0.4	NT

* = Virus titre in Log_{10} $TCED_{50}$ per swab
NT = Not tested

TABLE 6.10 ANTIBODY TITRES ($Log_{10}SN_{50}$) IN GOATS INFECTED WITH THE RBKO VIRUS

Animal	DAYS AFTER INOCULATION		
No.	7	14	21
G1	1.9	NT	NT
G2	2.3	NT	NT
G16	2.9	NT	NT
G19	2.9	NT	NT
G24	1.6	3.8	4.0
G29	1.9	NT	NT

FIGURE 6.1 SMALL EAST AFRICAN GOATS.

FIGURE 6.2 ATAXIA IN A GOAT INFECTED WITH RBKO VIRUS.

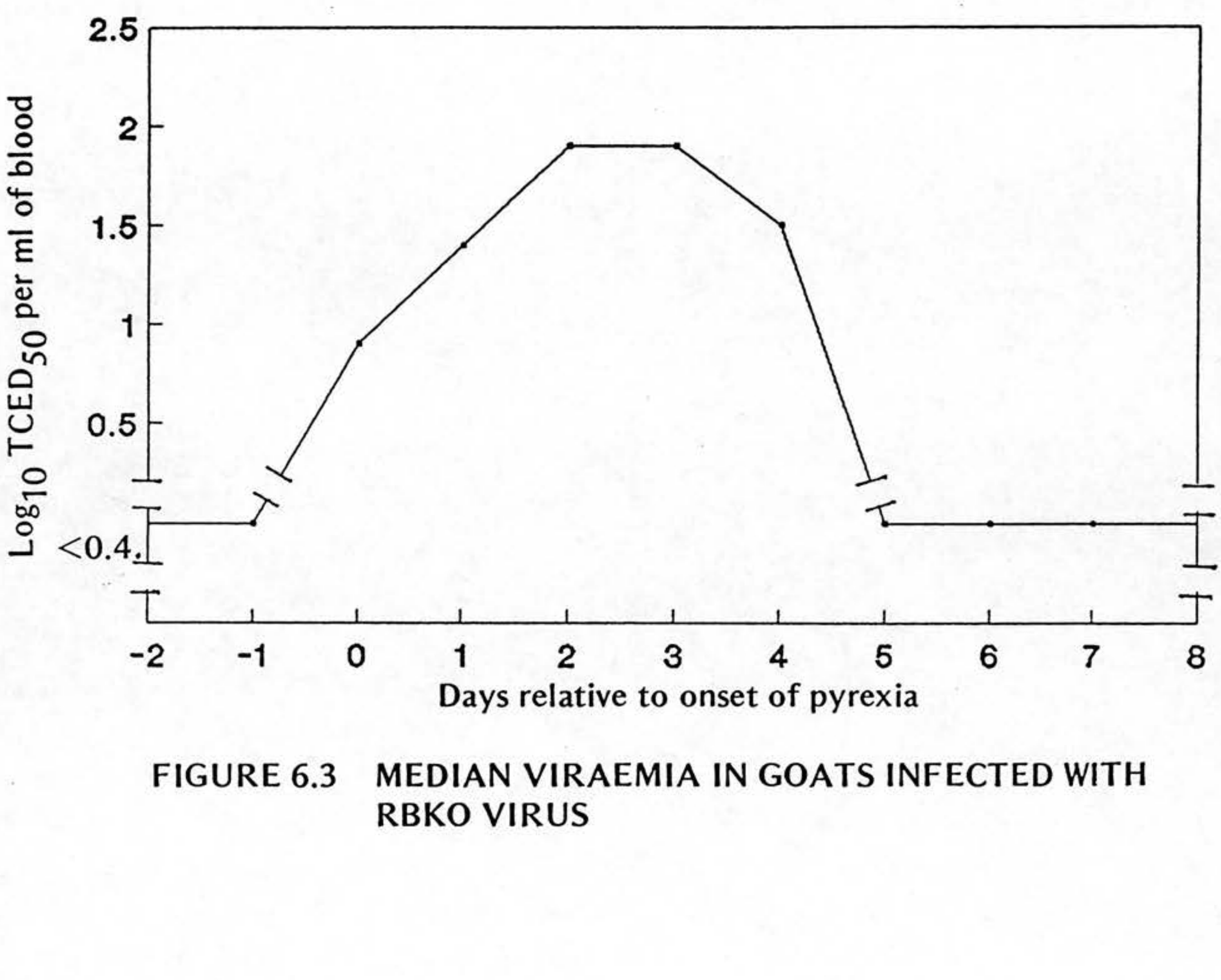

FIGURE 6.3 MEDIAN VIRAEMIA IN GOATS INFECTED WITH RBKO VIRUS

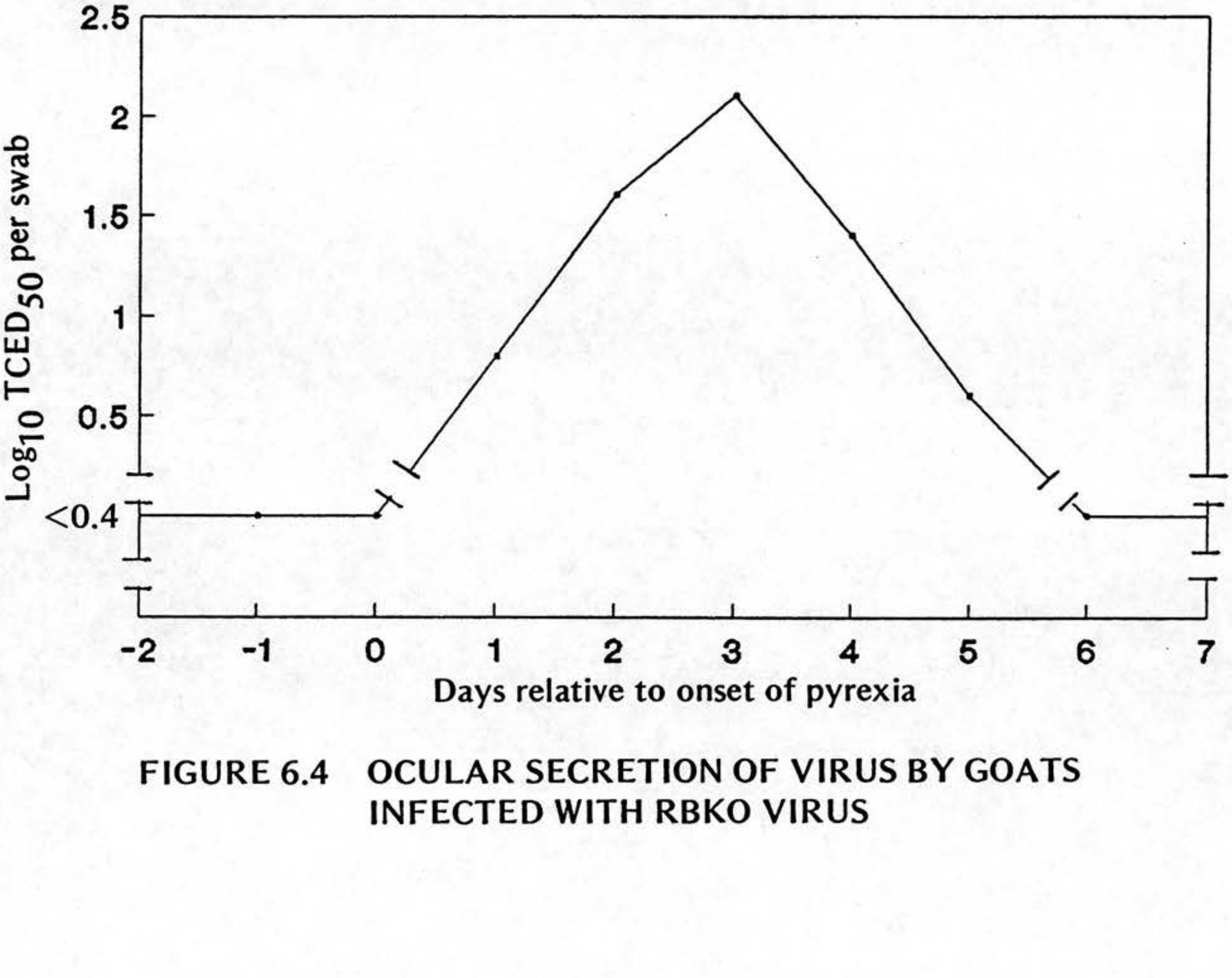

FIGURE 6.4 OCULAR SECRETION OF VIRUS BY GOATS INFECTED WITH RBKO VIRUS

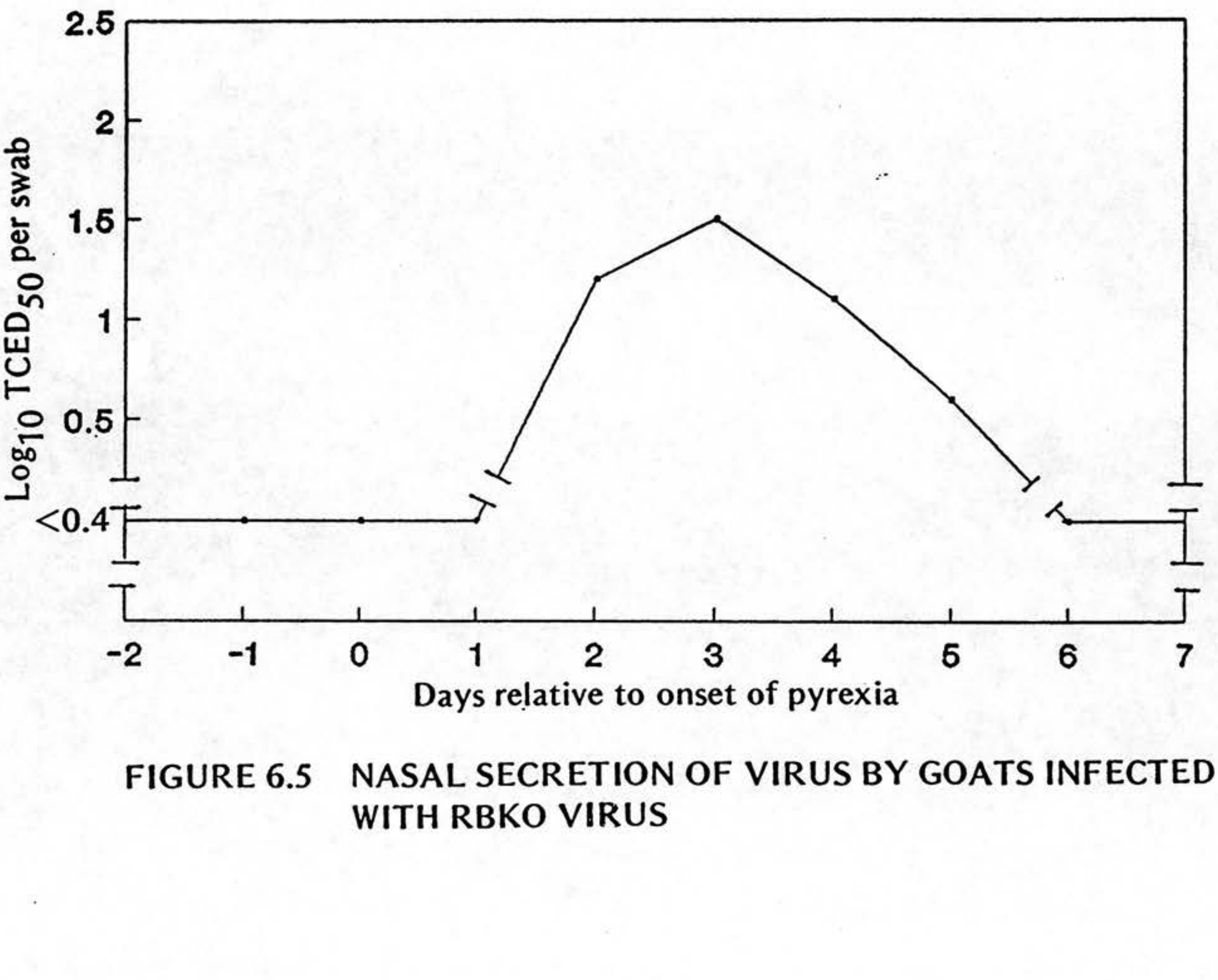

FIGURE 6.5 NASAL SECRETION OF VIRUS BY GOATS INFECTED WITH RBKO VIRUS

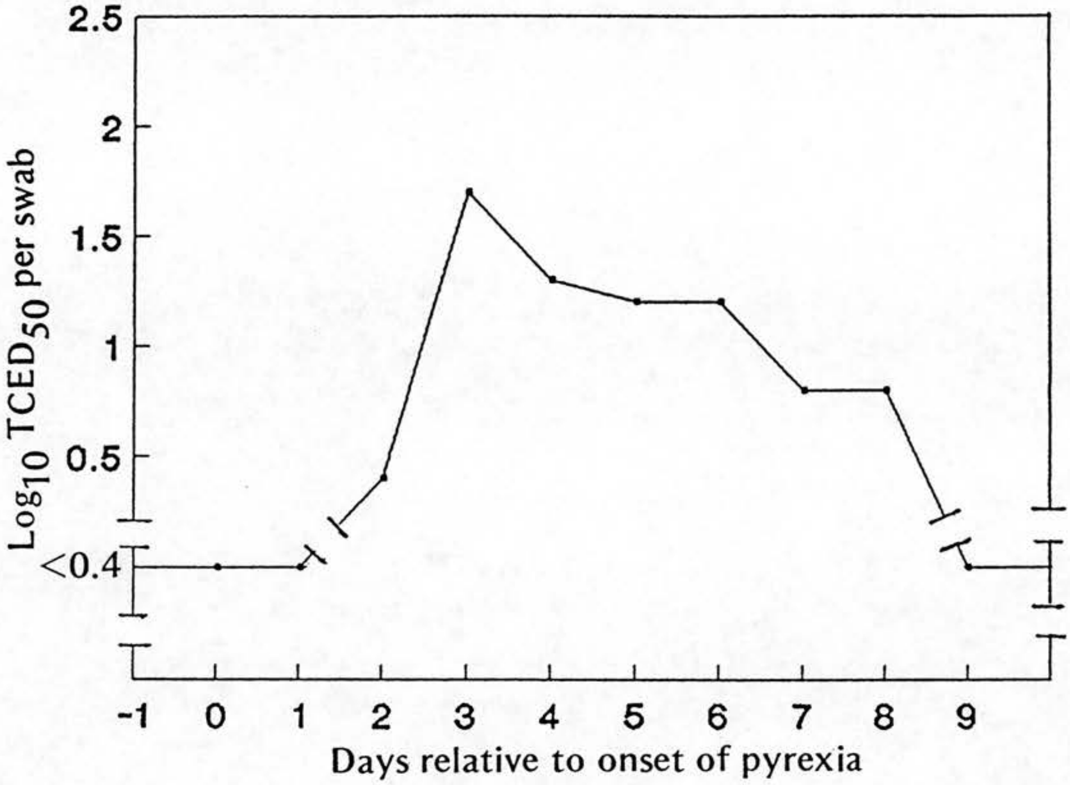

FIGURE 6.6 VAGINAL SECRETION OF VIRUS BY GOATS INFECTED WITH RBKO VIRUS

CHAPTER SEVEN

RESPONSES OF PREGNANT RABBITS TO INFECTION WITH RINDERPEST VIRUS

INTRODUCTION

Exposure of rabbits to ox strains of rinderpest virus does not produce recognisable clinical signs (Scott, 1964). Exposure of these animals to 'rabbit-adapted' rinderpest virus, however, induces pronounced fever and pathognomonic lesions in the lymphoid tissues (Cheng and Fischman, 1949). The significance of these signs has long been recognised and resulted in the exploitation of rabbits as a model for experimental studies on rinderpest and its causative virus (Yamanouchi et al, 1974 a,b).

The Nakamura III strain of lapinized rinderpest virus has been associated with abortion in cattle (Kesteven, 1949; Scott, 1963) and claimed to be capable of transplacental transmission in rabbits (Chawla and Sinha, 1961).

The effect of rinderpest virus on the rabbit foetus is not known. The present investigations were carried out to determine the clinical and virological effects of rinderpest virus infection in pregnant rabbits and the possible utilization of the rabbit as a model for investigations on the response of pregnant farm animals to infection with rinderpest virus.

MATERIALS AND METHODS

Cell cultures

Bovine kidney cells

BK cells were prepared by the method of trypsinization as previously described (Chapter Two).

Vero cells

Vero cells were propagated and maintained as previously described (Chapter Six).

Virus strain

The Nakamura III lapinized strain of rinderpest virus stored at the National Veterinary Research Centre, KARI, Muguga was used. It consisted of a freeze-dried suspension of infected rabbit spleen and mesenteric lymph nodes in infected rabbit blood (Rampton, Evans and Scott, 1958).

Experimental rabbits and their inoculation

Four- to five-month-old New Zealand White does were mated and their mating dates recorded. Non-pregnant rabbits of the same breed and age were used in sub-inoculation experiments with aborted foetal rabbit tissues. The rabbits were kept in pairs in cages and fed on kale and rabbit pellets and provided with water *ad libitum*.

Six rabbits, 10 days pregnant were each inoculated intravenously with 1 ml of a 20 per cent suspension of the lapinized rinderpest virus. Another group of 6 rabbits was similarly infected when 20 days pregnant. A third group of 6 rabbits was left as an uninfected control group. All the animals were examined daily for clinical signs of disease and rectal temperatures were recorded every morning.

Collection and processing of samples

Rabbits were bled for serum prior to inoculation with virus and at weekly intervals thereafter. Aborted foetal blood, lung, spleen, thymus and, where possible, foetal mesenteric lymph nodes were collected soon after abortion. Similar samples were also collected from new-born rabbits soon after birth.

Tissues from each animal were pooled, homogenized in a mortar and pestle with PBS to give a 20 per cent tissue suspension. One ml of the suspension was inoculated IV into two susceptible rabbits. BK and Vero cells in roller tubes were simultaneously inoculated with ten-fold dilutions of the 10 per cent tissue suspension. Inoculated animals and cultures were examined for the development of clinical rinderpest and seroconversion and viral CPE respectively.

Antigen detection

Aborted foetal thymus, spleen, lung and lymph nodes were examined for the presence of rinderpest precipitating antigen using the agar gel immunodiffusion test.

RESULTS

Clinical signs

Only 8 of the 12 inoculated rabbits exhibited signs of illness. The incubation periods ranged from 2-4 days, the median being 3 days (Table 7.1). Peak fevers were attained on the days of their onset and the median duration of pyrexia was 3 days (Table 7.1). Fevers were accompanied by respiratory distress and anorexia which lasted 3 to 4 days in the majority of the rabbits. Slight serous oculo-nasal discharges were observed in 20 per cent of the reactors from the day of onset of fever and lasted 2-3 days. Diarrhoea which lasted 2-4 days developed in 2 rabbits 2 days after the onset of fever (Table 7.1)

Six of the 8 reacting rabbits aborted 2-4 days after the onset of fever (Table 7.2) and of the 6, 3 died between 2 and 6 days after abortion. One of the remaining 2 reacting rabbits, R5, died on the 5th day after the onset of fever without aborting and the other, R10, recovered without aborting (Tables 7.1 and 7.2). The 4 rabbits that did not sicken did not abort, did not die and kindled normally at term.

Virus isolation and antigen detection

Rinderpest virus was not isolated in BK and Vero cell cultures from tissue homogenates of rabbit foetuses recovered post-mortem or after abortion. Similarly rinderpest virus antigens were not detected in foetal rabbit tissues in the AGID test. However, precipitating antigens were not demonstrated in spleen and mesenteric lymph nodes of aborted and live born rabbits and in tissue homogenates or exudates from the rabbits that died.

Control rabbits

No clinical disease, abortion or development of antibodies to rinderpest virus were demonstrated in the non-infected control rabbits and in the inoculated rabbits that failed to react clinically. The rabbits kindled normally 30 $\pm$ 1 days after mating.

Clinical signs in subinoculated rabbits

Rabbits inoculated with 20 per cent tissues homogenates from rabbit foetuses recovered post-mortem or following abortion or normal kindling did not develop clinical signs of rinderpest.

Serology

The serological response of rabbits which recovered from infection with the lapinized strain of rinderpest virus is shown in Table 7.3. Low amounts of antibody were first detected on the 7th day of inoculation and high titres of above 3.0 $\log_{10}SN_{50}$ had developed by the 3rd week after inoculation. Neutralizing antibodies were not demonstrated in the rabbits that were inoculated with foetal tissue homogenates and in two of those that failed to react clinically.

Pathology

The only significant post-mortem change observed in the rabbits that died from the disease was an enlargement of the lymphoid tissues in the Peyer's patches, the appendix and sacculus rotundus. The lymphoid tissue was chalk-white in colour and stood out prominently from the rest of the surrounding tissue. Aborted foetuses appeared slightly hyperaemic below the skin.

DISCUSSION

With the exception of the abortions, the clinical and pathological responses of the rabbits to infection with the lapinized strain of rinderpest virus were similar to those previously described (Cheng and Fischman, 1949). In the present study 4 out of 6 rabbits infected at 10 and 2 out of 6 rabbits infected at 20 days of pregnancy aborted between 2 and 4 days following the onset of fever i.e. during the acute phase of the disease. In contrast, abortion was not observed in the non-infected control rabbits. Neither rinderpest virus nor virus antigen was demonstrated in the tissues of the rabbits that died or from the aborted foetuses. Chawla and Sinha (1961) reported the demonstration of rinderpest virus in lapine foetuses obtained post-mortem from infected pregnant does. The present study has not confirmed the transplacental transmission of rinderpest virus in pregnant rabbits.

The failure to demonstrate infectivity and virus antigens in aborted foetuses may mean that the abortions occurred too early in the acute stage of the disease before transmission of virus to the foetus had taken place. The abortions were therefore probably due to disturbances in maternal health and or placental function.

TABLE 7.1 CLINICAL RESPONSES IN RABBITS INFECTED WITH LAPINIZED RINDERPEST VIRUS

Animal No.	Gestation age (days) when infected	Incubation period (days)	Days of fever	Diarrhoea	Deaths or Recoveries
R1	10	3	2	-	5D
R2	10	NR	-	-	-
R3	10	3	3	+	6R
R4	10	2	2	-	6R
R5	10	4	3	-	5D
R6	10	2	3	-	8D
R7	20	NR	-	-	-
R8	20	NR	-	-	-
R9	20	2	3	+	5R
R10	20	4	2	-	4R
R11	20	3	3	-	6D
R12	20	NR	-	-	-

D = Died on indicated day
NR = No reaction
R = Recovered on indicated day

TABLE 7.2 ABORTION IN RABBITS INFECTED WITH LAPINIZED RINDERPEST VIRUS

Animal No.	Clinical results	Abortion	Day* aborted	Day* of death	Foetal recovery _in situ_	Liveborn
R1	+	+	3	5		-
R2	-	-	-	-		+
R3	+	+	2	-		-
R4	+	+	4	-		-
R5	+	-	-	5	+	-
R6	+	+	2	8		-
R7	-	-	-	-		+
R8	-	-	-	-		+
R9	+	+	4	-		-
R10	+	-	-	-		+
R11	+	+	3	6		-
R12	-	-	-	-		+

* = Day after the onset of fever

TABLE 7.3 ANTIBODY TITRES ($Log_{10}SN_{50}$) IN RABBITS INFECTED WITH LAPINIZED RINDERPEST VIRUS

Animal No.	Days after inoculation 7	14	21
R1	2.0	NT	NT
R2	<0.3	<0.3	<0.3
R3	1.8	2.4	3.1
R4	2.4	2.6	2.9
R5	1.6	NT	NT
R7	1.3	NT	NT
R8	1.3	1.9	NT
R9	2.1	2.7	NT
R10	2.0	2.1	NT
R11	1.6	NT	NT
R12	<0.3	<0.3	NT

NT = Not tested

CHAPTER EIGHT

GENERAL DISCUSSION AND CONCLUSIONS

The early reports by Aldige (1918) and Banerji and Mohan (1935), that rinderpest virus is capable of causing abortions in cattle and goats have been confirmed and extended. In addition, abortion was observed in rabbits infected with rinderpest virus although the report that transplacental transmission of the virus occurred in rabbits (Chawla and Sinha, 1961) was not confirmed. In this respect rinderpest virus closely resembles the viruses of human measles and canine distemper, its fellow members in the Morbillivirus genus. To-date no reports of similar abortions in goats and sheep have been recorded in relation to infection with the virus of peste des petits ruminants.

There is evidence obtained retrospectively that the virus of measles crosses the placental barrier in pregnant women and infects the foetus at different stages of gestation (Blattner and Heys, 1961, Jespersen, Littaner and Sagild, 1977)). The ensuing foetal infection leads to the birth of defective children, abortion and developmental anormalies including mental retardation, congenital heart disease and hare-lip (Dyer, 1940; Packer, 1950). In addition, endometritis, cervicitis and abortion have been described in rhesus monkeys infected with measles virus (Renne, McLaughlin and Jenson, 1973).

In dogs, Krakowka, Confer and Koestner (1974) have reported transplacental transmission of canine distemper virus (CDV) by demonstrating virus specific antigens in tissues of gnotobiotic pups obtained from naturally infected bitches. In another study, Krakowka, Hoover, Koestner and Ketring (1977) examined the effects of experimentally induced CDV infection in pregnant dogs. Abortion occurred in one bitch on the 7th day of infection but no evidence of foetal infection was obtained. The second bitch developed a subclinical infection and gave birth to three CDV-infected pups. Further clinical, virological and immunological studies showed CDV infection in another group of gnotobiotic pups produced at term by a naturally infected bitch. Krakowka and others (1977) concluded that canine distemper should be added to the list of transplacental infectious diseases in the dog.

In this study abortions in cattle were influenced by the virulence of the infecting virus strain but not by the gestational age at which it took place. Four virus strains of different origin and passage histories were employed, all having been selected on the basis of their virulence for cattle. The original selection criterion was later supported by statistical analyses of the clinical parameters observed in infected cattle (Appendix 2). Using Kruskal-Wallis and Mann-Whitney tests, for example, significant differences were detected in clinical responses to the four strains making it possible to differentiate them (Appendices 2, 3 and 4). Thus based on the days of fever, two virus groups, one consisting of the KAG and RBKO strains and the other of the RBT/1 and RBKK strains were defined (Appendices 3 and 4). The placing of the RBKO strain together with the KAG strain was attributable to the fact that most of the animals infected with the RBKO virus died very early in the acute stage of the disease.

Analysis of peak fevers resulted in the KAG and RBT/1 strains emerging as one group while the RBKK and RBKO strains appeared as a separate group (Appendices 3 and 4). The average fever values also placed the KAG and RBT/1 strains together and the RBKK and RBKO strains as a separate group. Examination of the clinical signs scores without fever gave 3 significant subsets, two of which overlapped viz: KAG-RBT/1 and RBT/1-RBKK. The third subset contained only RBKO strain (Appendices 3 and 4).

More telling however were the results of the analysis of the total clinical scores including the fever parameter. These clearly defined the strains into three groups i.e. the KAG, the RBT/1 and the third group consisting of the RBKK and RBKO strains (Appendices 3 and 4). The RBKK virus was at this level separated from the RBKO strain only on the basis of their different mortality rates i.e. 12 and 75 per cent respectively. The latter grouping of the strains confirmed the criteria used to select the strains for this study as shown in Table 2.1 which defined each virus separately.

There was also a relationship between abortion and severity of clinical signs. The strains that produced moderate to severe clinical signs also resulted in abortion but not those that produced very minimal clinical signs. The RBT/1 and RBKK viruses were more important causes of abortion than were the attenuated KAG and the highly virulent RBKO strains. The practical implication is that in the field, abortions in cattle are more likely to be encountered in high-risk areas where susceptible animals with a relatively high innate resistance become exposed to moderately virulent virus strains spilling over from endemic areas. Such abortions are less likely to occur in endemic situations where most adult animals are immune from previous natural exposure or vaccination and in virgin soil epidemics in which most cows die from the acute disease.

The gestational age at which infection takes place influence the outcome of many viral infections of cattle. The virus of BVD, for example, can infect a pregnant cow and depending on the age of gestation may cross the placenta and cause no ill-effects in the foetus or it may lead to a variety of clinical syndromes including foetal death, abortion, still-births, teratogenic defects or, more importantly, it may establish a persistent infection (Duffell and Harkness, 1985). The latter outcome only occurs when the gestation age is less than 90 days.

Following a series of experimental infections of pregnant cattle with rinderpest virus, Jacotot (1931) reported that abortions occurred when animals were infected in mid or late gestation. The data in the present studies support Jacotot's observations and further show that the stage of gestation has no influence on the outcome of abortion in cattle when infected after 100 days gestation. No attempt was made to establish the effect of the virus on the foetus before the establishment of a competent immunological system. Nevertheless two foetuses from cows X3 and X106 were probably immunologically incompetent when exposed at about 3 months of age to the RBKK virus. One foetus, from cow X3 was aborted and showed antigenic evidence of _in utero_ infection. The calf from cow x106 was born normal at term, and had no evidence of transplacental infection with rinderpest virus. These preliminary findings suggest that persistently infected antibody negative calves are not part of the pathogenesis of rinderpest virus. However, more work is necessary to confirm this suggestion.

The immunological maturity of a foetus at the time of infection is of major significance in many viral infections (Schultz, 1973). In BVD infection for example, infections early in foetal life lead to the development of a persistent virus infection in many tissues and organs and the birth of a calf which remains infected for life. This state appears to arise from an inability of the foetus to recognise viral antigens as "non-self". In consequence the foetus fails to mount a normal immune response and when born the calf is indefinitely without antibody to the virus and remains seronegative. Such calves continuously excrete large quantities of virus thereby transmitting infection to susceptible in-contact individuals in the herd. Almost all foetuses are capable of mounting an antibody response to BVD virus by 126 days of gestation (Duffell and Harkness, 1985). It is rare however to observe antibody production by the foetus before 90 days gestation. Between these ages, the response varies with the individual foetus and its immunological maturity. The lack of information on the existence of cattle persistently infected with rinderpest virus may indicate that such infections do not occur or, if they do, they are extremely rare.

Abortion may occur in cattle as a direct effect of viral infection or can result indirectly through changes in maternal body temperature or placental function caused by placentitis (Ragsdale _et al_, 1948; Barlow, 1972). The causal role of the virus can be established by experimental infection followed by abortion and the confirmation of the presence of the virus, virus specific antigens and antibodies or histological changes in the foetus or placenta. The detection of virus and virus precipitating antigens in aborted foetuses from cattle respectively infected with the RBKO and RBKK viruses indicated that transplacental infection of the foetus had occurred and this probably led to foetal death and expulsion. There were erosive mucosal lesions in one foetus aborted by RBKK virus infected cow. This adds to the evidence of transplacental infection by rinderpest virus. It is known in addition, that the epitheliochorial cotyledonary-type placenta in the bovine does not permit passage of maternal immunoglobulins to the foetus (Brambell, 1970). The occurrence of neutralizing antibodies to rinderpest virus in the pre-colostrum serum of newborn calves from cows infected with the RBT/1 and the RBKK strains, therefore, provide further supportive evidence of an in utero foetal infection with rinderpest virus.

The possibility that the virus induced pathological lesions in the placenta thus leading to leakage of maternal antibodies into foetal circulation cannot be ruled out. It is however doubtful that the high antibody levels observed in the foetuses were solely due to such leakage.

The majority of abortions in cattle in which there was virological and antigenic evidence of foetal infection occurred between the 2nd and 7th weeks after recovery from the disease. The presence of virus in the bovine uterus several weeks after recovery is of considerable epidemiological significance because movement of such infected animals from one area to another poses a risk of transmitting infection.

All the foetuses aborted during or soon after the acute stage of the disease were devoid of infectious virus, virus antigens and antibodies. In addition a few of the foetuses aborted after the 2nd week of maternal recovery had no evidence of *in utero* infection. No reasons could be ascribed to these abortions. It is possible that the foetuses died soon after infection and before any significant virus multiplication had taken place. It may also be that the virus isolation and detection techniques employed were not sensitive enough. On the other hand foetal death and expulsion may have resulted from changes in maternal health such as high fever or damage to the placenta.

Studies in mice have shown that fever *per se* may harm the foetus. MacFarlane, Pennycuik and Thrift (1957) showed that pregnant rats resorbed their foetuses when kept at 30^{o}C. Ragsdale and others (1948) observed abortion in 2 pregnant cows following 27 hours exposure to a temperature of 100^{o}F. It would be reasonable therefore, to assume that the high fever which occurs in rinderpest infections can cause severe physiological changes in the body and thereby lead to abortion.

Maintenance of an intact pregnant state depends upon a healthy functional placenta. Disturbances in placental health and function are therefore likely to lead to foetal death and expulsion. Studies in sheep have shown that infection of pregnant ewes with the virus of Border disease may cause a placentitis which among other syndromes, can lead to foetal expulsion (Barlow, 1972). There was evidence of gross pathological changes in the placentomes of some of the aborted foetuses indicating the development of a placentitis in rinderpest infected cattle.

The reason for attempting to establish the time of onset of foetal infection with rinderpest virus was to provide an understanding of the role of the placenta in the infection and the pathogenesis of the disease in the foetus. Although the time was not clearly defined, there was an indication that this varied considerably with the virulence of the infecting virus strain. While infection of the foetus with the moderately virulent RBKK strain occurred after the disappearance of the virus from maternal circulation, infection with the highly virulent RBKO strain was, in contrast, demonstrated during the viraemic phase in the dam. This variation may probably be due to differences in the invasiveness of the two strains.

Attempts to demonstrate contact transmission of infection from aborting to susceptible cattle were not successful. One major reason for the failure was that most of the cows either aborted during or soon after the acute phase of the disease or did not abort but gave birth to normal calves at term. There was serological evidence of *in utero* infection in four of the live born calves. Virus was not detected in the aborted foetuses although it is possible that it might have been present in quantities too low to be detected by the cultural techniques used or to be transmitted to the in-contact susceptible steers. Another speculation is that there just was no viable virus in the foetuses. One reason for the latter speculation is that virus inactivation might have occurred due to autolytic changes in the foetus. Although most of the expelled foetuses appeared grossly normal, a few appeared pathologically affected. It was not possible to establish the time that elapsed between foetal death and expulsion.

It is claimed that putrefaction and autolytic changes associated with lactic acid fermentation in rinderpest infected carcasses rapidly destroy the virus (Edwards, 1925; Daubney, 1928; Curasson, 1932). Any considerable lapse in time between foetal death and expulsion would be expected to enhance foetal putrefaction thereby leading to virus inactivation and hence its absence in foetal tissues.

There is no information on the thermal response of the bovine foetus to infection with rinderpest virus. It would however, be reasonable to assume that after the establishment of a functional nervous system, the bovine foetus would respond to rinderpest virus infection with a rise in body temperature, probably to a magnitude comparable with that in adult cattle. Rinderpest virus is relatively heat sensitive and is rapidly destroyed within hours when subjected to temperatures above 37^{o}C (Scott, 1959a). Occurrence of high temperatures in an enclosed uterine environment for a few hours would most likely result in virus destruction and absence of infectivity in foetal tissues and fluids.

Although there is controversy over the effects of putrefaction (White, 1958) and heat (White and Cowan, 1962) on rinderpest virus precipitating antigen, positive results have been obtained with lymph nodes collected from decomposed carcasses (Scott and Brown, 1961) and after exposure of lymph node extracts to heating at 100^{o}C for 30 minutes (Ishii <u>et al</u>, 1964). This could explain the presence of rinderpest virus antigen in aborted foetal tissues in the absence of infectious virus.

The median prevalence of abortion following the field outbreak of rinderpest in Kenya was 33 per cent with most animals aborting 2 to 4 weeks following clinical recovery from the disease. These findings confirm those of Layard (1757) that some cows "with calf at the critical time of this disease may only give signs of such abortion and bear their calf several days, nay even weeks before they slip it, and then recover". While in West Africa, Aldige (1918) observed that "in cows which recovered natural rinderpest infections, abortion occurred much less often during the height of the clinical disease, many occurred during the period of convalescence but most were noted after complete recovery". Jacotot (1931) reported foetal expulsion between 21 and 86 days following experimental inoculation of pregnant cattle with serum-virus mixtures i.e. approximately 2-11 weeks after clinical recovery. The present observations on the experimental and natural disease are in agreement with these early reports. The prevalence of abortion in the field was slightly lower than that in laboratory infections with the same virus. It must be emphasised that data on the field disease were provided by stock-owners and herdsmen and the number of the affected animals may not be accurate.

Although Aldige (1918) observed numerous abortions in recovered cows the incidence rate in field outbreaks of rinderpest has not been reported before. The results from the Kenyan outbreak show that the rate was high enough to cause concern. There is, in addition, the problem of a prolonged presence of the virus in the herd beyond the documented quarantine period of 10-21 days (Gamgee, 1866) following the death or recovery of the last case of the disease and also the worrying possibility of its transmission to other susceptible stock long after the quarantine imposition has been lifted. It is imperative therefore that further investigations be carried out to determine the possibility of virus transmission from aborting to susceptible in-contact animals using different virus strains and also to establish the epidemiological significance of such infections on the current rinderpest control policies and eradication strategies.

Very limited studies have been conducted to establish the effect of rinderpest virus in pregnant rabbits and goats. In the present work two strains of the virus induced many abortions in pregnant goats. Similarly a lapinized strain of rinderpest virus induced abortion in pregnant rabbits. There was however no evidence of direct virus involvement in the causation of abortion in these species. The mechanisms that triggered the abortions in these species were thus not established but could have been due to disturbances in either maternal health or placental function. Abortions occurred in most animals during or soon after the acute phase of the disease. According to Cedric Mims (1968) "there is no virus known which infects the placenta but not the foetus, and leads only indirectly to foetal damage". Dr. Mims' conclusions were based on observations of viral infections in man and it is not established whether a similar situation holds true in ruminants and rabbits. The RBKO virus strain was recovered from the placentomes of infected goats and even though attempts to isolation virus from aborted foetal tissues were unsuccessful, the abortifacient effects of the virus were probably exerted at the placental level. Whether similar mechanisms operated in goats infected with the KAG virus strain and in rabbits exposed to the lapinized virus strain cannot be ruled out.

There are several classic descriptions of peste des petits ruminants in goats (Braide, 1981, Scott, 1981, Taylor 1984) but none mentions abortion as a clinical syndrome of the disease except Durtnell (1972) who studied a similar disease in Red Sokoto goats in Nigeria. Abortions have recently been observed in pregnant goats in Nigeria following vaccination with tissue culture rinderpest vaccine in the wake of a field outbreak of PPR (Taylor, personal communications). However, it was not established whether the abortions were due to infection with PPR or the vaccine although it is doubtful that the latter would cause abortion in goats. It would be interesting to establish the effects of PPR virus on a developing caprine foetus.

CONCLUSION

Virulent strains of rinderpest virus were shown to be capable of causing abortion in pregnant cattle, goats and rabbits. It was further shown that abortion in these species may occur both in the first and second half of gestation. While abortions in goats and rabbits occurred during or soon after the acute stage of the disease, in cattle most abortions took place 2 to 7 weeks after the disappearance of the clinical signs of the disease. Transplacental infection was demonstrated in cattle infected with virulent but not vaccine strains of rinderpest virus. Infectious virus and virus precipitating antigens were mainly detected in foetuses from cows that aborted from 10 days after recovery.

Although contact transmission of infection from aborting to susceptible cattle was not established, it would be prudent to assume that it may occur in a large cattle population undergoing a moderate infection from a moderately virulent strain of virus. Further work is required.

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APPENDIX 1 ESTIMATION OF VIRUS TITRES IN SAMPLES AT UNDILUTED INOCULAR ($10^{0.0}$) USING SPEARMAN-KÄRBER METHOD

Number of tubes	Number positive	Transformed titre (per ml)	Back transformed titre (per ml)
5	5	$10^{2.4}$	$10^{1.4}$
5	4	$10^{2.0}$	$10^{1.0}$
5	3	$10^{1.8}$	$10^{0.8}$
5	2	$10^{1.6}$	$10^{0.6}$
5	1	$10^{1.4}$	$10^{0.4}$
5	0	$<10^{1.4}$	$<10^{0.4}$

APPENDIX 2 CLINICAL PARAMETERS IN PREGNANT CATTLE INFECTED WITH DIFFERENT STRAINS OF RINDERPEST VIRUS

Animal No.	Virus strain	Days of fever	Peak fever	Diarrhoea days	Diarrhoea severity	Mouth lesions days	Mouth lesions severity	Abortion	Death
Z793	KAG	4	40.0	-	-	-	-		
Z794	"	-	39.4	-	-	-	-		
Z795	"	3	40.1	-	-	-	-		
Z796	"	2	39.8	-	-	-	-		
A13	RBT/1	4	40.0	-	-	2	+		
A14	"	5	40.1	-	-	-	-	+	
A18	"	5	40.2	-	-	3	++	+	
A20	"	5	40.0	-	-	2	+		
X 3	RBKK	12	41.1	6	+	3	++	+	
X 19	"	5	40.6	-	-	3	++	+	
X 93	"	4	41.0	3	++	2	+	+	
X 94	"	6	40.8	-	-	4	++	+	
X106	"	5	40.0	-	-	3	+		
X110	"	4	40.4	-	-	1	+	+	
Y15	"	7	41.0	3	+	3	++	+	
Y57	"	5	40.5	5	++	2	+	+	
B1	"	4	40.2	-	-	3	+	+	
B2	"	4	39.8	2	+	3	+		
B3	"	5	40.2	2	+	2	+		
B4	"	3	40.8	1	+	4	++		+
B5	"	8	41.7	-	-	4	+	+	+
B6	"	3	40.3	2	+	3	+		
B7	"	*	40.6	-	-	2	+	+	
B8	"	*	39.9	4	+	3	++	+	
B9	"	5	40.3	-	-	4	+		
Z413	RBKO	2	41.0	-	-	1	++		+
Z414	"	5	40.5	2	++	3	+++++	+	
Z418	"	2	40.6	-	-	-	-		+
Z420	"	2	41.5	-	-	1	++		+

* = Records not available

APPENDIX 3 MEDIANS OF CLINCIAL PARAMETERS

Parameter	VIRUS STRAIN KAG	RBT/1	RBKK	RBKO	Kriskal-Wallis H statistic
Days of fever	2.5	5.0	5.0	2.0	10.622*
Peak fever (^{o}C)	39.9	40.0	40.5	40.8	12.251**
Average morning fever (^{o}C)	39.8	39.9	40.2	40.5	13.079**
Clinical score without fever	0	2	6	21	16.988***
Total score	5.95	13.45	23.40	36.45	16.128**

* P <0.050 ** P <0.010 *** P <0.001

APPENDIX 4 SIGNIFICANT SUBSETS OF CLINICAL PARAMETERS

Clinical parameter	Number of subsets	Subsets	Median
Days of fever	2	RBKO -KAG	2
		RBT/1-RBKK	5
Peak fever	2	KAG -RBT/1	$40.0^{o}C$
		RBKK -RBKO	$40.6^{o}C$
Average morning fever	2	KAG -RBT/1	$39.8^{o}C$
		RBKK -RBKO	$40.3^{o}C$
Clinical score without fever	3	KAG -RBT/1	0
		RBT/1-RBKK	5
		RBKO	21
Total score	3	KAG	6
		RBT/1	13
		RBKK -RBKO	26

Preliminary observations on rinderpest in pregnant cattle

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A Kabete 'O' strain of rinderpest virus enhanced in virulence was inoculated subcutaneously into four cows which were between six and eight months pregnant. All the cows developed clinical signs of rinderpest from the third day after inoculation and shed high titres of virus in their ocular and vaginal secretions during the course of the clinical disease. Three of the cows died of rinderpest on the third day after the onset of fever but no virus was isolated from their fetuses which were examined post mortem. The fourth cow showed complete clinical and virological recovery by the eighth day after the onset of fever and aborted an eight-and-a-half-month-old fetus on the 12th day after it recovered. Rinderpest virus was demonstrated in a wide range of the aborted fetal tissues. Virus was also detected in the maternal vaginal discharges up to 24 hours after abortion. The only gross pathological change observed was a severe necrotising placentitis.

THE pathogenesis of rinderpest virus infection in cattle has been extensively investigated (Plowright 1964, Taylor and others 1965). Several workers have recorded abortion in cattle after natural or experimental exposure of pregnant cows to rinderpest virus (Layard 1757, Sorrell and Cater 1912, Jacotot 1931, Scott 1963). Jacotot (1931) in addition demonstrated, by subinoculation into susceptible calves, the presence of virus in the blood of two of 17 aborted fetuses and in the vaginal fluids and lochia alba in one of three aborting cows.

However, no systematic studies have been undertaken to provide quantitative data on the dissemination and replication of the virus in the reproductive organs of infected cattle, particularly in pregnant cows. The availability of cell cultures for the isolation of rinderpest virus from infected animal tissues (Plowright and Ferris 1962) makes it possible to reexamine these problems in a more systematic manner. This report records the results of the inoculation of pregnant cows with a Kabete 'O' strain of rinderpest virus at a low cell culture-passage level.

Materials and methods

Cell cultures

Primary bovine kidney cell cultures were prepared as described by Plowright and Ferris (1959a). For virus isolation, the cells were used as primary monolayer cultures in roller tubes (Plowright and Ferris 1962), while for serology they were employed as secondary cultures after dispersal and suspension in Eagles' minimum essential medium (MEM; Wellcome Reagents) containing 10 per cent ox serum. The cells were seeded at a concentration of 2×10^5/ml.

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Viruses

A virulent Kabete 'O' strain of rinderpest virus was injected at its fifth passage level in bovine kidney cells (Plowright and Ferris 1959b) into a susceptible steer. The spleen from the reacting steer was harvested and stored frozen until required, an aliquot being titrated in cattle. A high cell culture-passage strain of the virus was used in serological tests after 99 passages in bovine kidney cells.

Experimental animals and their infection

The experimental animals were six to eight months pregnant *Bos taurus-Bos indicus* crosses, aged three to five years. Each cow was inoculated subcutaneously with 2 ml spleen suspension containing an infectivity titre of $10^{5 \cdot 2}$ cattle median infectious doses (ID50) of the low-passaged virulent Kabete 'O' strain of rinderpest virus. The animals were examined daily for clinical signs of disease.

Collection and processing of samples for virus isolation

Blood samples in ethylenediamine tetra-acetic acid disodium salt (EDTA), and without anticoagulant, together with ocular and vaginal secretions, were collected daily from the cows. Fetal thymus, lung, kidney, spleen, liver, testis, abomasum, meconium, placentomes, membranes and fluids were collected at necropsy or from the aborted fetus.

Buffy coats were separated from the EDTA blood, and 10-fold dilutions inoculated into bovine kidney tube cultures and rolled at 37°C overnight. After three washes and changes of medium, the cultures were again rolled at 37°C and examined for 14 days for the development of viral cytopathic effects.

Solid tissues were homogenised in a sterile mortar and pestle as 10 per cent suspensions in sterile phosphate buffered saline (pH 7·2). Tenfold dilutions of each homogenate were inoculated on to five- to seven-day-old roller tube monolayer cultures and subsequently treated like the blood leucocyte inoculated cultures. Ocular, nasal and fetal fluid samples were similarly diluted and virus adsorption allowed to take place for 90 minutes at 37°C before changing the medium.

Virus neutralisation tests

Tests for virus neutralising antibodies were carried out in microtitre plates (Rossiter and Jessett 1982) using serum samples collected weekly for five weeks. The virus neutralisation titres were calculated by the method of Spearman-Kärber (Lennette and Schmidt 1964).

Results

All four cows developed fever together with congestion and hyperaemia of the nasal, ocular and oral mucosae three days after inoculation. Salivation and mucosal erosions were evident one day later and were severe in two of the cows including the only one that recovered. Three cows died on the third day after the onset of fever without exhibiting diarrhoea. The fourth cow which had a transient blood-tinged diarrhoea, recovered within eight days of the onset of clinical signs.

The cow which recovered manifested marked straining and developed an odourless straw coloured vaginal discharge on the morning of the 12th day of recovery and expelled an eight-and-a-half-month-old dead fetus four hours later. No other signs of abnormality or clinical disease were observed in the cow after the abortion and it continued to make a full recovery.

Maternal changes at necropsy

The dead cows had severe erosive and haemorrhagic gastroenteritis characteristic of rinderpest. There were necrotic foci and congestion in the nasal mucosae and signs of mild pneumonitis in the lungs. Although the gravid uteri appeared grossly normal, necrosis and congestion of the placentomes were observed in three of the four cows.

Fetal pathology

The fetuses obtained from the three dead cows at necropsy and from the cow which aborted appeared grossly normal. However, there were large quantities of blood tinged abdominal and thoracic fluid in the aborted fetus.

Virus isolation

Viraemia was first detected in all the cows one day before the onset of pyrexia and persisted until death or the fifth day after the onset of pyrexia in the survivor. The peak titre for viraemia in the latter cow was $10^{2\cdot5}$ median tissue culture infectious doses (TCID50) ml of blood, which was attained on the third day of pyrexia. Viraemia was never detected in this cow after the fifth day following the onset of fever.

Rinderpest virus was demonstrated in the ocular secretions from all the cows one day before the onset of fever, and in the vaginal secretions one day after the onset of fever. Virus was detected in increasing quantities in these secretions until death or the second day of fever in the survivor but declined steadily thereafter and was no longer detectable in these secretions by the fifth day.

Virus was detected in vaginal discharges immediately after the cow which recovered aborted and again 24 hours later.

Rinderpest virus was detected in the spleen, liver, abomasum, lung, meconium, chorioallantoic membranes and placentomes of the aborted fetus (Table 1). The titre of virus was highest in the placentomes ($10^{2\cdot5}$ TCID50/g) but ranged between $10^{1\cdot2}$ to $10^{1\cdot8}$ TCID50/g in other tissues. No virus was isolated from the fetal membranes, tissues and fluids from the fetuses of the cows which died.

Serology

Antibody development in the survivor was normal and appeared six days after infection and reached peak levels after 15 days. Neutralising antibodies were not demonstrated in the sera from the animals which died, and were not detected in the blood of the aborted fetus.

TABLE 1: Recovery of rinderpest virus from aborted fetal tissues

Fetal tissue	Virus titre*
Placentome	2·6
Spleen	2·0
Lung	1·8
Abomasum	1·8
Meconium	1·8
Liver	1·2
Chorioallantoic membrane	1·6
Kidney	1·2
Testis	0·0

*Log_{10}TCID50/g

Discussion

With the exception of the abortion and the vaginal excretion of virus the clinical and virological responses of the cows observed in the present study were similar to those already recorded in cattle inoculated with the low cell culture-passaged virulent Kabete 'O' strain of rinderpest virus (Plowright and Ferris 1959b, Mushi and Wafula 1984).

In the present study one of the four cows that developed rinderpest showed complete clinical and virological recovery from the disease by the eighth day after the onset of fever but aborted on the 12th day after recovery. High levels of rinderpest virus were demonstrated in a wide range of the aborted fetal tissues and maternal vaginal discharges. These findings are similar to the earlier reports by Jacotot (1931) that infection of pregnant cattle with rinderpest virus may lead to abortion and occurrence of the virus in fetal blood and maternal vaginal fluids.

The cessation of virus excretion in vaginal discharges after the fifth day after the onset of fever and its presence for only 24 hours at most after abortion would indicate that this virus was contained in fetal tissues and fluids and released in the reproductive tract at the time of abortion.

The failure to isolate rinderpest virus from fetal membranes and tissues collected post mortem from three bovine fetuses on the fourth day after the onset of viraemia would suggest either that at this early stage of infection very low and undetectable levels of virus were present in fetal membranes and tissues or that there was a late invasion and transmission of rinderpest virus from maternal tissues to the fetus.

The frequency and pathogenesis of rinderpest virus-induced abortion in cattle are not known and are currently under investigation.

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